

JOURNAL OF THE INSTITUTE OF BREWING

JANUARY—FEBRUARY, 1949

DYN-355/49 FI
24-3-49

EDITOR:

JULIAN L. BAKER, F.C.G.I., F.R.I.C.

Assistant Editor: I. A. PREECE, M.Sc., Ph.D., F.R.I.C.

ABSTRACTORS:

A. A. D. COMBIE, B.Sc., F.R.I.C.
C. RAINBOW, Ph.D.

I. A. PREECE, M.Sc., Ph.D., F.R.I.C.
T. J. WARD

Indian Institute of Technology, DELHI

PAGES

MONTHLY REVIEW—European Brewery Convention, 1; Type Cultures, 1; The Institute of Brewing Collection of Yeasts, 2; First International Congress of Biochemistry, 7; Report of the Berlin Brewing Experimental and Teaching Institute for 1947, 7; Annual Report of the Swiss Breweries Experimental Station, 8; The Sections: Scottish Section, 8; London Section, 9; Midland Counties Section, 10; Correspondence, 10; Obituary, 11.

THE INSTITUTE OF BREWING RESEARCH SCHEME—

The Growth and Fermentation of Yeast 6479 with Simple Peptides as Nitrogen Nutrients 13
By W. R. Damlé, M.Sc., and R. S. W. Thorne, Ph.D.

The Bios Requirements of some Top-Fermentation Brewery Yeasts in Relation to the Nitrogen Source 18
By R. S. W. Thorne, Ph.D.

A Study of Ropiness in Beer 26
By J. L. Shimwell, D.Sc., F.R.I.C.

Thirty-First Report on the Trial of New Varieties of Hops, 1947 29
By Prof. E. S. Salmon

LONDON SECTION—

From Cask to Consumer 38
By J. W. Scott

ABSTRACTS 47

REVIEWS 55

Printed by W. HEFFER & SONS LTD., CAMBRIDGE,
for THE INSTITUTE OF BREWING

(Temporary Office: The Goring Hotel, Grosvenor Gardens, London, S.W.1)

Malts of Distinction

make

Quality Beers

Telephone:
MIRFIELD 3322

Telegrams:
"Sutcliffe, Mirfield"

JOURNAL OF THE INSTITUTE OF BREWING

MONTHLY REVIEW

EUROPEAN BREWERY CONVENTION

CONGRESS AT LUCERNE IN MAY, 1949

THE European Brewing Convention which was instituted to promote scientific co-operation in the Malting and Brewing industries will hold its second Congress at Lucerne from 29th May to 3rd June, 1949. There will be four scientific sessions at which the principal papers and discussions will be concerned with (1) proteins in brewing; (2) the production of sterile beer for sale by means other than pasteurization. In order to promote full discussion, the papers presented will be printed and circulated before the Congress to those who have signified their intention to attend. In addition to the scientific meetings and the general meeting of the Convention, visits to breweries, excursions and a banquet have been arranged. Membership of the Congress is open to all scientific and technical members of the industries. The subscription to the Congress is 100 Swiss francs (about £5 17s.); this entitles the subscriber to a copy of the Congress proceedings, containing the papers as circulated before the Congress and to the final report containing addenda to the papers and the discussions. Subscriptions for ladies accompanying members will be 15 Swiss francs. Accommodation will be arranged in three of the largest hotels in Lucerne at approximately 25 francs daily, including breakfast and one other meal.

Both programme and application forms for membership are now obtainable from the Institute of Brewing, Goring Hotel, Grosvenor Gardens, London, S.W.1.

* * * *

TYPE CULTURES

At a Specialist Conference on Culture Collections of Micro-organisms held in London under Government auspices in August, 1947, it was announced that the National Collection

of Type Cultures, held by the Lister Institute for the Medical Research Council, was being reorganized, and that in future, most of the mould fungi would be housed with the Imperial Mycological Institute at Kew. The future of the yeast cultures was raised, and eventually the Institute of Brewing agreed to take over this collection.

These yeast cultures, numbering about 200, are in duplicate, each organism being kept as a dried culture over phosphorus pentoxide and in 10 *per cent.* sucrose solution. Pending the time when the Institute's research laboratories can maintain these cultures they have been distributed among the laboratories of six or seven breweries in different parts of the country, which have the necessary facilities. The organisms are being kept on an agar made up with unhopped beer wort of gravity 1040, and will be sub-cultured every three to four months. The great majority of the cultures have developed successfully, although the originals were several years old, but it is not possible at this stage to guarantee their purity, since the present temporary curators have undertaken simply to maintain the cultures passed to them. No doubt when the Institute's laboratories are able to take over the full collection, the possibility will be considered of checking the organisms.

For the present, the numbers given to the organisms in the National Collection of Type Cultures will be retained, but the collection will be known in future as the "Institute of Brewing Collection of Yeasts." A list is given below of the cultures held, and it has been re-arranged in alphabetical order. Individual cultures from this list, with fuller information regarding source, *etc.*, can be obtained by writing to the Secretary, The Institute of Brewing, The Goring Hotel, Grosvenor Gardens, London, S.W.1, who will forward the application to the appropriate laboratory. A small charge will be made for each culture.

THE INSTITUTE OF BREWING COLLECTION OF YEASTS

Catalogue

No.	Name of Organism.
2050	<i>Atelosaccharomyces</i> sp.
2811	<i>Brettanomyces</i> Claussen sp. Jørgensen strain.
2829	" " " Brussels strain.
2752	<i>Candida vulgaris</i> . Baarn.
2849	" " (Bon). Zoorpilz. Zikes.
477	<i>Debaryomyces globosus</i> . Carlsberg.
2059	" <i>kloeckeri</i> . Guill. and Péju.
2048	" <i>tyrocola</i> .
2056	" <i>tyrocola</i> Konokotine.
1871	<i>Endomycopsis fibuliger</i> . Ota 22 strain.
1908	" " var. <i>hordei</i> .
2045	" " var. <i>lindneri</i> .
2241	" <i>vernalis</i> . Baarn strain.
817	<i>Hansenia apiculata</i> . Lindn. Harden strain.
816	" " Schmitz. " "
478	<i>Hanseniaspora valbyensis</i> .
474	<i>Hansenula anomala</i> . Carlsberg.
1545	" " (Hansen) Syd. Chapman strain.
4621	" " Verona.
1546	" <i>belgica</i> (Lindn.) " " "
475	" <i>saturnus</i> . Carlsberg.
1834	" " 628 strain.
3965	Hill's Yeast.
4537	Hungarian Wine Yeast, Tokaj 22.
499	<i>Kloeckera africana</i> . Akbari 12 strain.
490	" <i>antillarum</i> . St. Thomas 208 strain.
2158	" <i>apiculata</i> . Chapman strain.
497	" " P.H. 4-2 "
492	" <i>austriaca</i> . St. Croix 409 strain.
485	" <i>corticis</i> . B. 60 strain.
491	" <i>germanica</i> . Harz 3 strain.
487	" <i>indica</i> . Himalaya strain.
498	" <i>javanica</i> . Java 104 strain.
494	" <i>jensenii</i> . Java 403 strain.
489	" <i>lafarii</i> . Java 105 strain.
1816	" <i>lindneri</i> . Java 395 strain.
484	" <i>malaiana</i> . Java 104a strain.

Catalogue

No.	Name of Organism.
496	<i>Kloeckera muelleri</i> . Java 201 strain.
486	„ <i>occidentalis</i> . St. Croix strain
493	„ <i>santacruzensis</i> . Alpes Autr. strain.
488	„ <i>willii</i> . St. Thomas 103 strain.
1639	Mahua Yeast. Fowler strain.
2146	<i>Monilia</i> sp. from Cutworm, No. 3.
2147	„ „ „ „ No. 4.
2755	„ <i>variabilis</i> . Baarn.
1849	<i>Mycoderma arborescens</i> . 1211 strain.
1848	„ <i>bordetii</i> . 1206 strain.
819	„ <i>cerevisiae</i> . Harden strain.
2055	„ <i>chevalieri</i> .
5023	<i>Mycotorula colostri</i> No. 156.
1846	<i>Nadsonia fulvescens</i> . 1146 strain.
2886	<i>Oidium lactis</i> Fres. strain 1.
2887	„ „ „ strain 2.
2887A.	„ „ „ strain 3.
3315	„ „ „ Wilkins strain.
481	<i>Pichia alcoholophila</i> . Carlsberg strain.
5021	„ <i>derossii</i> . Castelli 1935. No. 46.
3863	„ <i>hyalospora</i> .
479	„ <i>membranaefaciens</i> .
483	„ „ Carlsberg strain..
480	„ <i>polymorpha</i> . „ „
482	„ <i>suaveolens</i> . „ „
5022	<i>Pseudosaccharomyces magnus</i> Derossi 1920. No. 102.
2648	<i>Rhodotorula glutinis</i> . Baarn.
2628	„ „ var. <i>rubescens</i> .
2631	„ „ „ <i>rufula</i> .
1644	„ <i>minuta</i> Saito.
2622	„ <i>mucilaginoso</i> . Baarn strain.
2773	„ „ Castellani.
820	„ „ var. <i>sanguinea</i> . Harden strain.
2629	„ „ „ „ Harrison strain.
907	„ <i>plicata</i> . Chapman strain.
4585	„ <i>rubra</i> (Demme) Lodder.
241A.	„ <i>rutila</i> Harrison. Paine.
3864	<i>Saccharomyces anamensis</i> . Will and Heinrich.
3597	„ <i>blanchardii</i> .
1808	„ <i>brasiliensis</i> Lindner. 98 strain.

Catalogue

No.	Name of Organism.
7248	<i>Saccharomyces carlsbergensis</i> , A.T.C.C. 2345.
7014	" " 4228, A.T.C.C. 9080.
742	" " Carlsberg strain.
3865	" <i>cartilaginosus</i> .
815	" <i>cerevisiae</i> . Baker's yeast.
2160	" " Chapman strain.
6422	" " Fleischmann baker's.
5922	" " G.B. No. 354.
6420	" " Gebrüder Mayer.
466	" " Hansen Carlsberg.
381	" " Hansen 21.
4614	" " Hansen. Gebrüder Meyer.
7043	" " H.J.B.
5916	" " Kluyver.
6421	" " Old Process.
7239	" " NRRL 567.
6479	" " Thorne.
7157	" " Toronto.
2779	" " Top.
2054	" <i>chevalieri</i> .
3964	" <i>delbrueckii</i> .
2161	" <i>ellipsoideus</i> . Chapman strain.
467	" " Hansen 21. Carlsberg.
3866	" " Hansen var. cratericus.
1344	" " Michigan strain.
7040	" " No. 48 Michigan.
812	" <i>exiguus</i> (Reess). Harden strain.
3139	" <i>festinans</i> .
2303	" <i>fragilis</i> Jörgensen.
905	" " " Chapman strain.
1810	" <i>hansenii</i> . 112 strain.
1681	" <i>hansenii</i> Zopf. Carlsberg strain.
4536	" sp. Hungarian Wine Yeast. Badacsony I.
1809	" <i>ilicis</i> . 105 strain.
469	" <i>intermedius</i> . Hansen 21. Carlsberg.
5915	" " Kluyver.
5825	" <i>italicus</i> . No. 362.
1807	" <i>lactis</i> Dombrowski. 96 strain.
2053	" <i>lindneri</i> Guill.
472	" <i>marxianus</i> . Hansen 21. Carlsberg.
743	" <i>monacensis</i> . Carlsberg strain.
2049	" <i>muentzii</i> .
2159	" <i>pastorianus</i> . Chapman strain.
468	" " Hansen 21. Carlsberg.

Catalogue

No.	Name of Organism.
1071	<i>Saccharomyces pyriformis</i> . "Californian Bees" strain.
3519	" <i>saké</i> . Gray strain.
1015	" " Ling strain.
3212	" " Yabe. Chapman strain.
791	" <i>thermantitonum</i> . Berlin strain.
792	" " Chapman strain.
790	" " Jörgensen strain.
2105	" " Original strain.
471	" <i>turbidans</i> . Hansen 21. Carlsberg.
4203	" <i>turbidans</i> . Hansen. Berlin.
470	" <i>validus</i> . Hansen 21. Carlsberg.
1811	<i>Saccharomycodes ludwigii</i> . 113 strain.
473	" " Hansen. Carlsberg.
814	<i>Saccharomycopsis capsularis</i> . Harden strain.
6969	Sauterne Yeast. Pasteur.
1014	<i>Schizosaccharomyces mellacei</i> . Ling strain.
2502	" <i>octosporus</i> . Jörgensen.
902	" <i>pombé</i> . Chapman strain.
476	<i>Schwanniomyces occidentalis</i> . Carlsberg.
886	<i>Torula</i> sp. 2.
2630	" <i>aclotiana</i> Kufferath.
2635	" <i>aerius</i> . Saïto.
2639	" <i>alactosa</i> . Kluyver.
2638A.	" <i>aurantiaca</i> . Delft strain.
2647	" <i>aurea</i> Saïto.
2633	" <i>colliculosa</i> . Harrison strain.
2052	" " Hartmann strain.
2625	" <i>corallina</i> Saïto.
1302	" <i>cremoris</i> . Cream strain.
4481	" <i>dattila</i> Kluyver.
2626	" <i>fermentati</i> . Saïto.
2643	" <i>flava</i> . Saïto.
2640	" <i>galactosa</i> Kluyver.
2634	" <i>gropengiesseri</i> . Periplaneta strain.
2636	" <i>heveanensis</i> .
4185	" <i>kefyr</i> (Beij). Kluyver.
4207	" " Silber.
2637	" <i>lactosa</i> .
2624	" <i>lipolytica</i> Jacobsen.
2632	" <i>pulcherrima</i> .
2627	" <i>rubra</i> Saïto.
2649	" " "
2642A.	" <i>rugosa</i> . Delft strain.

Catalogue

No.	Name of Organism.
2673	<i>Torula</i> sp. Hansen. Hilderbrand strain.
2231	„ sp. Pink. Throat strain.
2232A.	„ sp. „ 1442.
2674	„ sp. Sax strain.
2046	<i>Torulaspora fermentati</i> .
2051	„ <i>rosei</i> Guill.
5864	<i>Torulopsis bacillaris</i> D.
2645	„ <i>holmii</i> .
1847	„ <i>laurentii</i> . 1204 strain.
2646	„ <i>lipofera</i> .
1303	„ <i>sphaerica</i> . Cream strain.
6593	„ <i>utilis</i> var. <i>major</i> Thaysen and Morris.
2078	Yeast (Bottom yeast).
2404	„ (Californian Wine).
5824	„ (Cheese Hayes).
5823	„ (Cheese Whittington).
3959	„ Fernbach 1 strain.
3960	„ „ 33 „
3961	„ „ 38 „
3962	„ „ 40 „
3963	„ „ 41 „
903	„ (Frohberg).
6462	„ Hybrid K. 471.
904	„ (Kefir).
607	„ (Lactose fermenting). Thaysen.
2442	„ (Leather). Marriott 2.
3341	„ (Logos). Chapman.
909	„ „ Ling strain.
4919	„ (Melbourne No. 1).
1467	„ (Mineral "Futterhefe")
5333	„ No. 4.
1346	„ (Pink). Olive strain.
3966	„ Race V.
2074	„ (Rasse II).
2031	„ (Rasse XII).
906	„ (Saaz). Chapman strain.
608	„ (Sternberg "675").
6594	„ sp. 61.
806	„ sp. (Wine yeast. Johannesburg II). 76 strain.
5827	<i>Zygopichia chiantigiana</i> No. 370.
901	„ <i>farinosa</i> . Chapman strain.

Catalogue

No.	Name of Organism.
813	<i>Zygosaccharomyces barkeri</i> . Harden strain.
2047	" <i>bisporus</i> Naganishi.
5826	" <i>florentinus</i> . No. 368.
4564	" <i>mellis</i> Fabian and Quinet D. I.
2057	" <i>nadsonii</i> Guill.
2058	" <i>pastori</i> . A. Guillermond.
4464	" <i>priorianus</i> Klöcker.

FIRST INTERNATIONAL CONGRESS
OF BIOCHEMISTRY

At the First International Congress of Biochemistry to be held at Cambridge from 19-25th August, 1949, in addition to the 11 sections already announced, it has been decided by the Organizing Committee to include a 12th section on Industrial Fermentations. This will include brewing, antibiotics, vitamins, solvents, yeast manufacture, etc., in their biochemical aspects. Mr. H. J. Bunker, M.A., of Messrs. Barclay Perkins, has been appointed Chairman of this section, and Dr. P. W. Brian, M.A., of Imperial Chemical Industries, Ltd., Butterwick Laboratories, The Frythe, Welwyn, Herts., has been appointed Hon. Secretary. Those interested in this section are urged to get in touch with Dr. Brian, and those who wish to present papers in this section should submit titles as soon as possible.

* * * *

REPORT OF THE BERLIN BREWING
EXPERIMENTAL AND TEACHING
INSTITUTE FOR 1947

THE Report is of special interest since it gives an account of the extraordinary difficulties with which German brewers are beset by reason of the acute dearth of all brewing commodities.

The Institute has leased an extensive farm in Rheinhessen where experimental barley growing is proceeding. Work has been carried out on disease-resistant varieties and on X-ray induced mutants.

The Chemical Section has been called on extensively to give advice on problems connected with the use of substitute raw material such as molasses, starch sugars and spent grains, for brewing, and also on the production of a beer-like drink from whey. Most German malting barley is not available for brewing, being exported in exchange for

essential foodstuffs. Only a small quantity is permitted for beer brewing.

Since the main bulk of beers produced were brewed from 3 *per cent.*, or even weaker, worts, it is not surprising that the help of the Biological Section has been sought with increasing frequency. Biological instability has been due, not only to the extreme thinness of the beers but also to the lack of fuel, which minimized sterilization by heat and prevented the application of low cellar temperatures. In addition, brewing had to be carried out sporadically, with long time intervals between brews, so that it has become impossible to carry over pitching yeast through more than 6 to 10 brews, owing to serious infection being picked up. Beers were brewed at relatively high O.G. and subsequently diluted with hopped water. The rapid onset of spoilage often followed this dilution, so that the highest degree of biological cleanliness was necessary, especially as far as concerned the water used for the breaking down of worts and for yeast washing. The situation was further aggravated by the low degree of attenuation (about 60 *per cent.*) usually obtained, the poor bittering power of the available hops, and relatively high p_H . The Institute was able to supply the demands for pure cultures of yeast and also supplied specially trained cultures of the lactose-fermenting *S. fragilis* for use in the preparation of whey "beer." The latter is a hopped drink, its preparation creating special problems of its own, with regard to hopping, fermentation and storage.

Other activities of the Institute are described in the Report. Thus, the Biochemical Section was investigating the possibility of using breweries for the preparation of other products, such as pharmaceutical and dietetic foods, during periods of lack of brewing materials when breweries are perforce inactive. The Engineering Section has carried

out advisory work on numerous brewery problems. The Report concludes with an account of the teaching activities of the Institute. Courses were run for brewery technicians, engineers and for practical brewers, together with short courses on engineering, on brewery economy and on soft drinks.

It is welcome news that certain of the Institute's publications, such as *Bier in Zahlen*, are already in print again, and hopes are entertained that the *Wochenschrift für Brauerei* will shortly reappear.

* * * *

ANNUAL REPORT OF THE SWISS BREWERIES EXPERIMENTAL STATION

The year 1947-8 has been one of great activity, the 6,820 investigations carried out being only slightly fewer than the highest of pre-war years. This work has been divided between the Chemical and Biological Divisions of the Station and the Report gives statistics, which may be compared with figures quoted for corresponding work in previous years.

The work of the Chemical Division has centred mainly on barleys, malts, hops and beers, but investigations on fuels (oil, coal, peat), soft drinks, malt extracts, spent grain, fruit juices, water, pitch, filter pulp, and other materials have also been dealt with. Most of the barleys were of Danish origin. Moisture content averaged 14.72 *per cent.*, with protein 10.91 *per cent.* and extract 78.75 *per cent.* on dry matter. Germinative energy and capacity were high in all cases but one. The Pilsner malts examined came mainly from American sources and gave an average extract of 79.58 *per cent.*, 0.69 *per cent.* lower than last year. Munich malts were 1.04 *per cent.* lower than 1946-7 at 78.51 *per cent.* extract and also had a lower average index of modification and diastatic power. Hops were chiefly Bohemian and showed α -acid and bittering power to be higher than in any year since 1943, excepting 1944. The original gravity and alcohol content of beers showed an all-round increase. Thus, pale lager beers were brewed this year from worts of 9.85 *per cent.* concentration, as against 9.16 *per cent.* for 1946-7, the corresponding figures for dark lagers being 9.82 and 9.26 *per cent.* respectively.

The bulk of the work of the Biological Division has been occupied with waters, worts, beers, pitching yeasts and sediments. A small deterioration of biological purity of pitching yeasts has been shown, 3.9 *per cent.* of the yeasts examined being unsatisfactory, compared with 2.2 *per cent.* last year. Vat beers showed a higher degree of infection, but beers on tap were slightly improved. In both cases, "sarcina" was the main contaminant. Statistics showed that infection of pitching yeasts and beers is highest in the autumn season. Keeping qualities of beers were somewhat lower than in 1946-7, the turbidities developed being mainly due to rod bacteria and "sarcinae," with some cases due to culture and wild yeasts.

* * * *

SECTIONS

SCOTTISH SECTION

At a meeting of the Section, held in the North British Hotel, Edinburgh, on 19th October, under the Chairmanship of Mr. L. Fletcher, a paper entitled "Scandinavian Brewing Plant and Methods" was given by Mr. K. H. Skinner, a member of the Section and first winner of an award under the John S. Ford Memorial Trust. The author, basing his remarks on his experiences in Denmark and Sweden during the tenure of the Award, presented numerous diagrams of Scandinavian equipment and plant lay-out, comparing these with British practice. From mechanized maltings there were discussed the cleaning system, vacuum drum driers, Saladin boxes and the Topf vertical kiln; costs were compared with those of the flooring system. Other processes dealt with included modifications of decoction mashing technique, mash filters, hot sludge separation by centrifugal and other methods, automatic temperature control and cleaning technique in plate-cooler installations, design of fermentation rooms, and details of centrifugal treatment of beer.

On 9th November, members of the Section were the guests of the Scottish Section of the Incorporated Brewers' Guild at a meeting in the North British Hotel, Edinburgh, with Mr. J. M. Wilson in the Chair. A paper was given by Mr. H. L. A. May, under the title

"Report on the 1948 Hop Crop." The influence of the weather on the crop at various stages of development was described and the crop prospects were discussed for the established varieties; the continued interest in certain of the new varieties was noted. In the prolonged discussion which followed, particular attention was devoted to marketing organization, storage problems, flavouring characters of new varieties, and antiseptic value.

At a meeting of the Section held in the North British Hotel, Edinburgh on 14th December, 1948, Mr. L. Fletcher in the chair, a paper entitled "Hop Growing" was read by Dr. A. H. Burgess, of Wye College. The author pointed out that the cultivation of hops in England is localized in certain areas of Kent, Sussex and Hampshire, in the South-East, and of Worcestershire and Herefordshire in the West Midlands. This localization of the crop is also found in other countries where hops are grown and is probably due to the particular climate of these areas rather than to a special type of soil, but is also due in some degree to the difficulty of procuring in other districts the skilled labour necessary for carrying out the numerous specialized operations involved.

A series of some 80 pictures were shown on the screen to illustrate work on hops throughout the year. These, in addition to indicating the large amount of labour expended on the crop, emphasized the constant attention and care which must be exercised by the grower to produce a well developed crop, free from damage by pests and diseases, and satisfactorily dried.

The discussion which followed concerned itself chiefly with the question of hop diseases and with problems introduced by the use of mechanical pickers.

It was announced that the following revised and additional syllabus arrangements had been made:

15th February: lecture by Dr. I. A. Preece.

17th/18th March: exhibition at the North British Hotel, Edinburgh of the 1948 growth "New Varieties of Hops."

17th March: "Hop Picking by Machine in America," Major Derek Wigan.

18th March: Annual Dinner.

LONDON SECTION

A meeting of the London Section was held at the Horse Shoe Hotel, Tottenham Court Road, London, W.1, on Monday, 13th December, at 6 p.m. Mr. B. M. Brown, Chairman of the Section, presided when a paper entitled "Diastatic Power and the Mash Tun" was read by Dr. I. A. Preece.

Dr. Preece reviewed work which has been carried out on the determination of α - and β -amylases in barley malts and on starch conversions brought about by mixtures of the two enzymes in controlled proportions. Methods for assessing reducing group production were also discussed, the high values obtained in presence of α -amylase by the ferricyanide method being noted. Malts of high D.P. tend to have a high content of α -amylase, but the proportion of $\beta : \alpha$ can be varied by the intensity of the kilning treatment. When the two enzymes act jointly on starch at p_H 5.4 and 149° F., the results obtained can be explained on the basis of four fundamental facts: (a) β -amylase is extremely unstable at the temperature of an infusion mash; (b) during its brief period of action, however, it brings about a relatively great degree of starch hydrolysis; (c) apart from a possible stabilizing effect of β - towards α -amylase, the actions of the two enzymes over a wide range are substantially independent in so far as the proportion of reducing groups liberated is concerned; (d) α -amylase activity is extremely slow beyond its inflection point, the level of which varies with the enzyme concentration. Thus, for the lowest D.P. values, a ratio of $\beta : \alpha$ of $2\frac{1}{2} : 1$ gives more fermentable reducing groups than higher ratios of the same D.P. value. Higher ratios ($3\frac{3}{4} : 1$ — $6\frac{1}{4} : 1$) give results which are scarcely distinguishable from one another at a given D.P., the fermentable reducing groups produced by such ratios exceeding those for $2\frac{1}{2} : 1$ when the D.P. is greater than 16. Susceptibility to temperature change in mashing is greatest with low D.P. values and with low $\beta : \alpha$ ratios, but β -amylase is apparently more stable, and α -less stable, in wort at mashing temperature than in dilute laboratory starch conversions at similar temperature.

A discussion followed the reading of the paper.

THE 45th Annual General Meeting of the London Section was held at the Horse Shoe Hotel, Tottenham Court Road, London, W.1, on Monday, 10th January, 1949, at 5.45 p.m.

The statement of accounts as presented by the Honorary Treasurer, was adopted and the election of office-bearers was carried out. As constituted for the ensuing year, office-bearers are as follows:—Chairman: Mr. B. M. Brown; Vice-Chairman: Commander A. Cory-Wright; Representative of the Section on the Council: Dr. J. H. Oliver; Representative on the Examinations Committee: Mr. W. J. Watkins; Committee: Mr. R. E. Barclay, Dr. L. R. Bishop, Messrs. W. N. Errington, J. V. Godfrey, A. J. Gough, M. S. Heycock, C. E. Lanfear, Dr. J. H. Oliver, Messrs. J. H. Parker, M. W. Plumptre, J. Tatton Ridd, W. A. Whitley, H. D. L. Williams, J. H. B. Wilmot and T. Wright. The Hon. Secretary and Treasurer is Mr. J. A. Burns.

On the conclusion of the business of the Annual General Meeting, the Chairman, Mr. B. M. Brown, introduced the lecturer for the evening, Mr. M. W. Plumptre, who spoke on the subject of "Park Royal Brewery."

Mr. Plumptre discussed the main features of the design and construction of Park Royal Brewery and described the buildings, plant and machinery which have been installed there.

A brief allusion was made to the process as practiced at Park Royal, and special features of design which had been introduced to ensure easy cleaning and effective sterilization of mains and vessels were described.

A comprehensive system of metering and fuel-user analysis, which had been installed for fuel economy purposes, was described, the installation of meters having resulted in considerable saving in fuel which more than justified their expense.

The lecture was illustrated by numerous slides of drawings and photographs taken during the construction and operation of the Brewery.

MIDLAND COUNTIES SECTION

At a meeting of the Section held on 20th January, under the Chairmanship of Mr. B. Wood, Dr. C. J. Virden read a paper entitled "The Design and Interpretation of Tasting Experiments," in which various types of tasting experiment were discussed. It was shown that in order that the reliability

of results could be assessed by statistical analysis, strict independence of one taster from another must be achieved.

Two arrangements were described whereby the quality of beer is assessed by drinking rather than tasting under conditions which simulate to some extent the conditions under which beer is consumed by the public. Only one of these methods is susceptible to statistical analysis of its results, but both methods can lead to valuable results. Examples quoted show that the results obtained by these drinking techniques are not necessarily equivalent to the results obtained by tasting. Neither do the two methods differ from one another in any regular fashion. It is suggested that the more reliable results are obtained by the drinking techniques although exploratory work may still be carried out by tasting.

* * * *

CORRESPONDENCE

16, SOUTHWARK STREET,

LONDON, S.E.1.

28th December, 1948.

THE EDITOR,

THE JOURNAL OF THE INSTITUTE OF
BREWING.

DEAR SIR,—The careful work and observations of Messrs. Kelly and Bremner on malt analysis technique can, I suggest, serve to emphasize the special difficulties which face the Malt Analysis Committee in unifying the different techniques of malt analysis which have developed over a period of time that is now nearly 100 years. It is very understandable that workers become attached to techniques which they have developed, or perhaps even in which they have been brought up. The result is seen in those cases where two techniques for the same estimation are published, although care has been taken to see that the results given by them can be identical. An illustration of this is shown by Messrs. Kelly and Bremner's preference for the mash in beaker technique in determining the extract, on which they make some useful comments relating to detail, but I should imagine that this is less widely employed than the flask technique, which latter it can be suggested is more likely to give reproducible results in less experienced hands.

The main purpose of this letter is to suggest that those who prefer to determine the cold-water extractives by weighing the extract made for the determination of diastase are not so much succumbing to a temptation as continuing a very old practice. Where this is done it is usually made clear by referring to the result of this estimation as the "matters soluble in cold water," whereas the Committee Method is reported as the "cold-water extract."

I am particularly grateful to Messrs. Kelly and Bremner for showing that the diastase determination can be carried out on the Committee Cold Water Extract using buffered starch. It is clear that either worker can, if desired, avoid making two separate cold mashes. This, I hope, will meet the suggestion that the preference for the older method of determination is a matter of laboratory convenience and not carried out so much for its greater diagnostic value.

Messrs. Kelly and Bremner rightly point out that the malt mill receives less attention than it deserves, but it is not clear from their comments whether they realize that there were, in fact, three distinct types of Seck mill, all of which are probably still in use, and that, most unfortunately, the English one is a copy of the oldest type.

Yours faithfully,

J. H. OLIVER.

* * * *

OBITUARY

WALTER ALFRED RILEY,

1879-1948

WALTER ALFRED RILEY, who died at his home at Southwold on 7th November, 1948, was born in Norwich in 1879. He was the eldest son of Walter Riley, head brewer of Morgan's Brewery Co., Ltd., Norwich, and received his education at Paragon House School in the same town. He inherited his father's strong personality as well as his ability and skill as a brewer. He was a man of character and determination and displayed an aptitude for industry and hard work which enabled him to attain the prominent position he held in later life. Before taking up brewing in this country he spent several years as a pupil in several Continental breweries, finishing as a pupil in Alfred Jørgensen's Laboratory in Copenhagen. On his return to

England he took a course of Brewing Chemistry with the late John Heron and remained with him for a short time, acting as his assistant, subsequently joining his father as under brewer at Morgan's Brewery Co. On the death of his father in 1918, he succeeded him as head and was appointed to the Board in 1926.

He had the misfortune to lose his leg at the commencement of his career, and it was probably this disability that had an influence on his life and restricted his activities to Norwich and his home, although it did not in any way deter him from making full use of his capabilities.

He was a keen supporter of the Operative Brewer's Guild, which later became the Incorporated Brewers' Guild, and undertook the editorship of its *Journal* in its early days and by his ability and skill succeeded in building it up into the valuable publication it has now become. He has contributed a number of papers to the Institute of Brewing, which are characterized by their originality and practical value. He was also the author of a textbook on brewery by-products, which is recognized as an authority on the subject and one which satisfied a long-felt want. He was a Life Member of the Institute of Brewing, acted as chairman of the London Section 1920, was a member of the Council from 1911 to 1916 and was made a vice-president in 1925. He acted as an examiner for the Institute Examinations from 1923 to 1931, while he was also an active member of the Publications Committee. In recognition of his untiring work and his long association with the Incorporated Brewers' Guild, he was honoured by being elected their president in 1938.

The Brewery was not capable of absorbing all the interests of a man of Riley's untiring energy and capabilities and he soon turned his attention to the conduct of the affairs of his native town. He was elected a member of the City Council, on which he served for 25 years, achieving the distinction of becoming Mayor in 1935-6. Apart from his other activities, the City of Norwich was largely indebted to him for the interest he took in the disposal of the City sewage and when he was made chairman of the Sewage Committee in 1929, his scientific training well fitted him for undertaking a scheme for the re-organization of the City sewage disposal works and he

was able to introduce a very efficient scheme to the Council in 1938, which was to cost £600,000. It was a great disappointment to him, however, when the outbreak of war prevented this scheme being carried out. During the war as chairman of the A.R.P. Committee, he was untiring in his efforts to make the City A.R.P.-minded and it was the irony of fate that an air-raid not only destroyed his own home, but also damaged the Brewery close by. He was an ardent Conservative, acting at first as deputy head of the Norwich Conservative Party and later as leader in 1939, when Sir Robert Bignold retired. He was made a Justice of the Peace in 1940 and became an Alderman in 1937.

His health began to fail soon after the war ended and he spent the last years of his life at his home at Southwold, where he had retired, and after a very active life, he died in his 69th year. He leaves a wife and three daughters, who survive him.

HAROLD HERON.

R. L. SIAU

1870-1948

RAYMOND LOUIS SIAU, who died on 20th December, 1948, came of an English-French family. He was educated at St. Paul's School and the Royal College of Science and

later became an assistant to the well-known diabetic expert, Dr. F. W. Pavey, F.R.S.

After taking Brewing Courses at Birmingham University Brewing School, under Professor Adrian Brown, and at Copenhagen, he joined the staff of W. Butler & Co., Ltd., of Wolverhampton, as head chemist, in 1903. In 1911 he became head brewer and held this post until he retired in 1945. He was a most loyal and devoted servant of the Company.

He was an original Diploma member of the Federated Institutes of Brewing, and was on the Council from 1910-14 and an examiner from 1923-26. He was also an examiner for the City and Guilds Examination in Brewing. In 1912-13 he was chairman of the Midland Counties Section of the Institute of Brewing and in 1921, chairman of the Birmingham Section of the Incorporated Brewers' Guild. He had many papers published in the *Institute Journal*.

Not only was he exceptionally well informed on all matters concerning brewing, malting, and the fermentation industries, but had a mind stored up with all sorts of curious information on every conceivable subject.

He was of a genial and sociable nature and took an active part in Freemasonry, having attained Grand Lodge rank.

He leaves behind him many friends in brewing and masonic circles who mourn his loss.

W. R. WILSON.

THE INSTITUTE OF BREWING RESEARCH SCHEME

THE GROWTH AND FERMENTATION OF YEAST 6479 WITH SIMPLE PEPTIDES AS NITROGEN NUTRIENTS

By W. R. DAMLÉ, M.Sc., and R. S. W. THORNE, Ph.D.

(Received by the Chairman: 13/11/47)

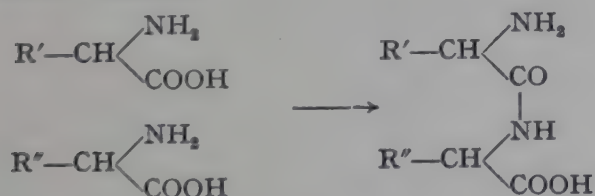
A considerable proportion of the nitrogen of beer wort is known to exist in the form of peptides; it is therefore important to know what value this class of compounds has as a source of nitrogen for yeast.

The following paper reports the results of an examination of the nutrient properties of some simple peptides in comparison with the related amino acids. It is shown that dipeptides tend to be perceptibly less efficient nutrients than free amino acids, and it also seems probable that their nutrient efficiency rapidly diminishes as the number of amino acid residues they contain is increased.

As regards the mechanism of their assimilation, they appear first to be hydrolyzed by yeast into their component amino acids which are then deaminated by the Ehrlich mechanism.

AMINO acids are usually considered to be the most important class of yeast nutrients present in wort; they constitute some 20 *per cent.* of the total wort nitrogen according to analyses made by Schryver and Thomas (this *Journ.*, 1929, 571). The nutrient values of a range of amino acids for a strain of *S. cerevisiae* (No. 6479 in the National Collection of Type Cultures) have previously been examined by one of us (*ibid.*, 1941, 255).

Peptides consist of two or more amino acids linked together in the following characteristic manner:—



They are called di-, tri-, tetra- or poly-peptides according as they consist of 2, 3, 4 or several amino acid residues. The amino acids in wort are the ultimate degradation products of the proteins originally present in the barley and are produced from them during the malting and mashing processes. The peptides in wort are incompletely degraded protein products formed during the same processes. They amount, according to Schryver and Thomas (*loc. cit.*), to some 30 *per cent.* of the total wort nitrogen, *i.e.*

a quantity exceeding that of the free amino acids of wort. In view of this relative profusion, their salient nutritive characteristics seem worth investigation.

Windisch, Kolbach and Hennecke (*Woch. Brau.*, 1928, 45, 389; this *Journ.*, 1928, 575) found that the assimilable nitrogen in wort amounted to nearly double the quantity of amino nitrogen (as estimated by formol titration or van Slyke determination) and furthermore they found that the higher peptones and proteins in wort were quite unassimilable by yeast. They therefore concluded that a considerable contribution to the assimilable nitrogen of wort is made by substances more complex than amino acids but less so than proteins or peptones, namely polypeptides. Thus the further question arises, at what stage of increasing complexity do peptides cease to be effective yeast nutrients?

Starting with 20 different amino acids, there are 400 (20²) theoretically possible dipeptides that can be formed from them, 8,000 (20³) tripeptides, and so on. Any investigation of the nutrient value of peptides must therefore be concerned more with classes than with individuals. Unfortunately, the commercially available peptides—with which most of the present work has been done—impose a severely limited choice.

Experimental.—Eight dipeptides have been examined: glycyl-*L*-leucine, *L*-leucylglycine, *dl*-alanylglycine, glycyl-*L*-tryptophane, *L*-leucyl-*L*-tyrosine, glycyl-*L*-tyrosine, glycylglycine and *D*-glutamyl-*D*-glutamic acid (see Note I); and one tripeptide: *L*-leucylglycylglycine.

The nutrient characteristics of these peptides were determined by supplying each of them as the sole source of nitrogen in a synthetic medium using the technique previously described (this *Journ.*, 1939, 14). Parallel comparison determinations were carried out with each of the constituent amino acids of the peptide under investigation, and also with an equimolecular mixture of the same amino acids. The latter control is considered the more significant one in view of the interactions which are known to occur when mixtures of amino acids are supplied to yeast (Thorne: *ibid.*, 1944, 186; 1946, 15). Yeast 6479, a top-fermentation variety of *S. cerevisiae* (*ibid.*, 1944, 222), was used for all the experiments.

Approximately 12 cultures were examined for each of the peptides and control media, and the mean results are given in Table I for each of the following growth characteristics:

r: the initial relative growth rate;

Y: the final yeast growth in grms. of moist yeast *per* litre;

F: the fermentation factor, relative to ammonium phosphate as unity;

N: the nitrogen uptake in grms. *per* litre.

The standard deviations for these characteristics are also specified at the foot of the table and represent an overall experimental error of ± 6 per cent.

DISCUSSION

Looking at the results first in a qualitative way, the following observations may be made:—

(i) Leucylglycine, glycylleucine, alanylglycine, leucyltyrosine, glutamylglutamic acid and glycylglycine tend to give growth characteristics not very different from those of the corresponding amino acid mixtures. (The "mixtures" corresponding to glycylglycine and glutamylglutamic acid are of course just glycine and glutamic acid respectively.) On the average the growth characteristics of these dipeptides are 10 per cent. less than those of the amino acid controls.

TABLE I
NUTRIENT CHARACTERISTICS OF PEPTIDES

							<i>r</i>	<i>Y</i>	<i>F</i>	<i>N</i>
peptides	Glycylleucine						1.75	10.1	0.66	0.168
	Leucylglycine						1.64	10.6	0.64	0.152
	Alanylglycine						1.72	9.0	0.63	0.119
	Glycyltryptophane						1.40	5.5	0.38	0.091
	Leucyltyrosine						1.58	9.8	0.67	0.145
	Glycyltyrosine						1.64	6.9	0.58	0.102
	Glycylglycine						1.53	1.5	0.40	0.042
	Glutaminyglutamic acid						1.63	12.7	0.80	0.242
	Leucylglycylglycine						1.37	4.6	0.42	0.063
controls	amino acids	Glycine					1.34	1.0	0.35	0.022
		Alanine					1.76	6.8	0.65	0.146
		Leucine					1.94	11.3	0.78	0.219
		Tyrosine					1.66	7.9	0.68	0.080
		Tryptophane					1.36	5.8	0.47	0.090
		Glutamic acid					2.37	12.3	0.99	0.226
	amino acid mixtures	Glycine + Leucine					2.03	10.5	0.75	0.187
		Glycine + Alanine					1.68	7.6	0.64	0.133
		Glycine + Tryptophane					1.30	1.9	0.37	0.046
		Leucine + Tyrosine					1.82	12.3	0.80	0.157
		Glycine + Tyrosine					1.32	1.6	0.32	0.040
		Leucine + 2 Glycine					1.45	6.3	0.53	0.121
			Standard deviation					±0.06	±0.5	±0.02

(ii) Glycyltryptophane and glycyltyrosine give considerably higher growth characteristics than the corresponding mixtures: r and F are about 25 *per cent.* greater while Y and N are 100–200 *per cent.* greater. The abnormal depressive effect upon yeast growth of glycine-tyrosine mixtures has already been described by one of us (*ibid.*, 1946, 15) and it appears from the results now obtained that glycine-tryptophane mixtures behave similarly. The growth characteristics of glycyltryptophane and glycyltyrosine only appear high in comparison with controls that are abnormally low; they are quite normal when compared respectively with tryptophane and tyrosine. Whatever may be the mechanism responsible for the inhibitive effect of these particular amino acid mixtures, it is evidently not operative when the corresponding dipeptides are used as nitrogen sources.

(iii) The growth characteristics of the tripeptide leucylglycylglycine are considerably less than those of the corresponding amino acid mixture, to the extent of about 25 *per cent.* If this can be regarded as a typical result for a tripeptide, one may construct a series:—amino acids, 100 *per cent.*; dipeptides, 90 *per cent.*; tripeptides, 75 *per cent.* . . . suggesting by extrapolation that tetrapeptides would be of seriously reduced nutrient value and higher peptides perhaps of very little use as compared with amino acids. As far as the evidence given here is concerned, such a conclusion must be extremely tentative, since not only is it based upon a single tripeptide, but that tripeptide, containing 2 glycine residues out of 3, cannot be regarded as the most typical representative of its class.

The mechanism by which yeast assimilates nitrogen from peptides may be discussed in the light of these results. There are two possibilities:—

(i) That peptide splitting enzymes in yeast first degrade the peptides into their constituent amino acids and that the yeast then assimilates nitrogen from these in the usual way. In this case it would be expected that the growth characteristics of any particular peptide would be closely correlated with those of the corresponding amino acid mixture.

(ii) That the yeast assimilates nitrogen from the free amino group of the peptide by

a process analogous to the Ehrlich reaction, leaving a residue still containing the characteristic peptide linkage, $-\text{CO}-\text{NH}-$. Here, no correlation would be expected between the growth characteristics of the peptide and those of the mixed amino acids. A correlation *might*, however, appear between the peptide characteristics and those of the corresponding amino acid containing the free amino group, *e.g.* between alanyl-glycine and alanine, glycylleucine and glycine, *etc.*

There is a standard statistical procedure, which need not be given here (see Note II), for calculating from such data as those of Table I the required correlations between the various factors, and of estimating the significance to be attached to the so-called partial correlation coefficients thus obtained. Table II gives the results of this analysis. It is seen that in no case is there any significant correlation between the growth characteristic of a peptide and the amino acid containing the corresponding free amino group. But there is (except possibly for r , the initial relative growth rate) a significant correlation between the growth characteristics of a peptide and those of the corresponding amino acid mixture. The weight of evidence is therefore in favour of the first of the alternative hypotheses formulated above, namely that peptides are first split into their component amino acids and then assimilated by yeast in the usual way.

It will be noticed that glycine is a constituent of all but two of the nine peptides which have been examined. Since glycine is, from the point of view of yeast nutrition, a somewhat exceptional amino acid the peptides containing it may also be suspected of being atypical. This is an advantage for achieving a clear distinction between the two groups of correlation coefficients considered above; more typical peptides might have shown less differentiation between these two correlations, making it more difficult to infer the mechanism of nitrogen assimilation from their behaviour. The atypical nature of these peptides is, on the other hand, a disadvantage in trying to assess the average nutrient value of peptides relative to amino acids, since they do not necessarily constitute a fair sample for furnishing an average.

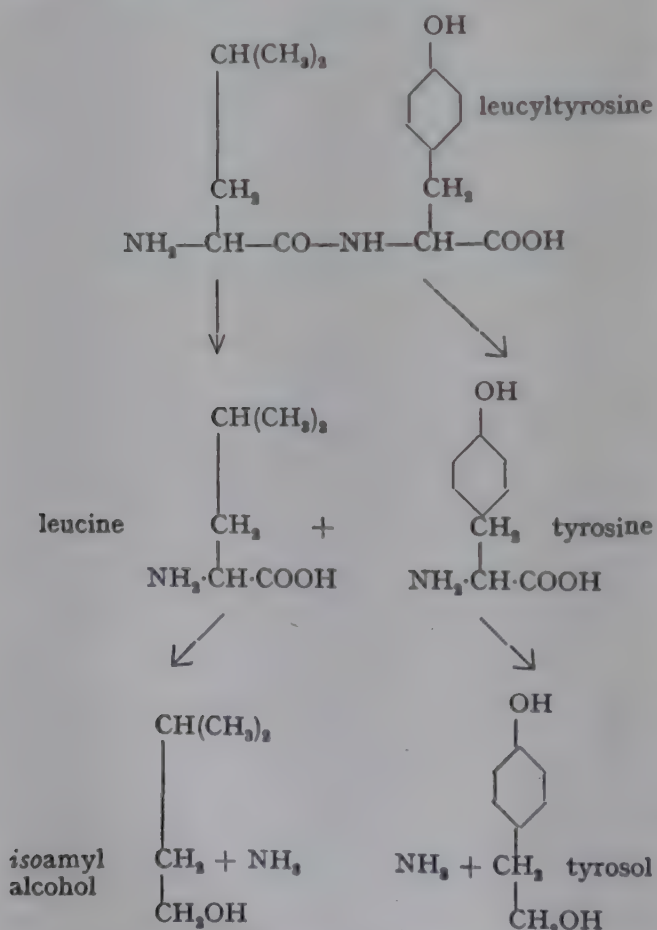
A more direct test of the hypothesis that peptides are split by yeast into their component amino acids which are then attacked

TABLE II

SIGNIFICANCE OF PARTIAL CORRELATION COEFFICIENTS BETWEEN GROWTH CHARACTERISTICS OF PEPTIDES AND CORRESPONDING AMINO ACIDS

		<i>r.</i>	<i>Y</i>	<i>F</i>	<i>N</i>
Partial correlation coefficient between:	Peptide and corresponding amino acid mixture	Doubtful	Significant	Significant	Very significant
	Peptide and corresponding amino acid containing free amino group	Not significant	Not significant	Not significant	Not significant

by the Ehrlich mechanism was carried out by using a suitable dipeptide as the sole source of nitrogen in a synthetic nutrient medium and then examining the fermented medium for the metabolic products of the peptide. Leucyltyrosine was chosen as a convenient peptide for this purpose since the ultimate end products which should be obtained on the above hypothesis, namely isoamyl alcohol and tyrosol, are easily identifiable. The expected reactions (Thorne: *ibid.*, 1937, 288) are as follows:—



The following nutrient medium was prepared:—

3,000 mls. standard mineral salt solution.
180 grms. sucrose.

5.25 grms. leucyltyrosine

0.03 gm. ferrous sulphate.

0.06 gm. inositol

6 mgrms. pantothenic acid.

0.003 mgrm. biotin.

0.15 mgrm. aneurin.

0.15 mgrm. pyridoxin.

The p_H of this medium was adjusted with soda to 5.5; its initial specific gravity was 1.025 and its total nitrogen content was 0.498 gm. After fermentation for 9 days with yeast 6479 at room temperature its specific gravity had fallen to 0.998 and its total nitrogen to 0.180 gm. The yeast crop weighed 46 grms. (moist). The uptake of nitrogen was therefore 0.318 gm. corresponding to 4.20 grms. of leucyltyrosine metabolised; this figure should probably be increased by about 10 per cent. to allow for the effect of nitrogen excretion (Thorne: *ibid.*, 1945, 6). On this basis the theoretical yields of isoamyl alcohol and tyrosol are 1.39 and 2.17 grms. respectively.

The filtered fermented medium was made alkaline with sodium bicarbonate and distilled *in vacuo* to a syrup. This was shaken with 250 ml. of 95 per cent. alcohol, filtered, and the filtrate evaporated on a water bath: the resulting syrup was taken up in 100 mls. of water and extracted continuously with ether for 3 days. The extract was dried with anhydrous sodium sulphate, filtered and evaporated. The resulting oil was cooled to crystallize, dried on a porous tile and recrystallized from chloroform. The tyrosol so obtained had m.p. 91° C. (theoretical 93° C.); yield 1.06 gm., i.e. 49 per cent.

The distillate from the fermented medium was extracted with ether for 3 weeks and the ether then removed from the extract after

drying with anhydrous sodium sulphate. The residual liquid was fractionally distilled, giving 0.94 gm. of a colourless liquid of b.p. 130° C. (b.p. of isoamyl alcohol is 130.5° C.). This substance was identified as isoamyl alcohol by preparing from it the esters of acetic and benzoic acids. A small excess of the material was boiled for 10 minutes with acetyl chloride or benzoyl chloride in a small distilling flask fitted with a reflux air condenser. The ester was then fractionated by distillation. Isoamyl acetate was obtained with b.p. 137°–138° C. (theoretical 139° C.) and isoamyl benzoate with b.p. 267° C. (theoretical 262° C.). The yield of 0.94 gm. of isoamyl alcohol is 68 per cent. theoretical.

Direct chemical evidence is thus provided for the view that peptides are split by yeast into their component amino acids which are then attacked by the Ehrlich mechanism. It might be mentioned that such a test can only provide a rigid proof when 100 per cent. yields of the expected end products are obtained; this is obviously unattainable in practice.

SUMMARY

The nutrient characteristics of a limited number of peptides have been examined. As far as typical behaviour can be inferred from the sample available, dipeptides appear to be only slightly inferior as nutrients to mixtures of the corresponding amino acids; the only tripeptide available was quite appreciably inferior. The data obtained support the view that the peptides are hydrolyzed by yeast to their component amino acids which are then assimilated by the normal mechanisms.

The authors' best thanks are due to Miss Doreen L. Birkett for assistance with the experimental work.

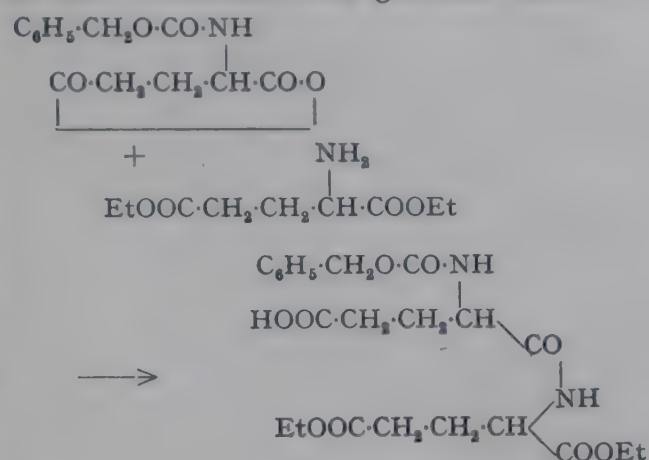
Institute of Brewing Research Laboratories,
University of Birmingham.

4th November, 1947.

NOTE I

Glutaminylglutamic acid was synthesized by the method of Bergmann and Zervas (*Ber.*, 1932, 65, 1192). Glutamic acid is treated with carbobenzoxy chloride, $C_6H_5 \cdot CH_2O \cdot CO \cdot Cl$, to protect the amino group; the anhydride of the carbobenzoxyglutamic acid is then prepared by treatment with

acetic anhydride. The resulting substance is then condensed with glutamic ester:—



The product is saponified and then reduced with hydrogen to regenerate the free amino group so giving glutaminylglutamic acid.

NOTE II

For any particular growth characteristic, e.g. final yeast growth, let $p_1, p_2 \dots$ represent the data for dipeptides, $m_1, m_2 \dots$ the data for the corresponding amino acid mixtures and $g_1, g_2 \dots$ the data for the corresponding amino acids with the free amino group. The correlation coefficient, r_{pm} , between p and m is given by:—

$$r_{pm}^2 = \frac{(\sum pm)^2}{\sum p^2 \cdot \sum m^2}$$

and similarly for r_{gp} and r_{mg} . The partial correlation coefficient, $r_{pm \cdot g}$, is a measure of the correlation between p and m after the effect of g has been eliminated and is given by:—

$$r_{pm \cdot g} = \sqrt{\frac{r_{pm} - r_{mg} \cdot r_{gp}}{(1 - r_{mg}^2)(1 - r_{gp}^2)}}$$

Similar expressions give $r_{mg \cdot p}$ and $r_{gp \cdot m}$.

Correlation coefficients are a precise measure of the amount of correlation between the variables in a set of data. They may vary over the range 1 to -1 . Values of 1 or -1 indicate complete correlation (direct or inverse respectively) between the variables while 0 signifies no correlation: the significance to be attached to intermediate values may be assessed from standard tables of values of the correlation coefficient for varying degrees of probability and varying sample sizes. The precise significance to be attached to a correlation coefficient is expressed in terms of probability:—A value

of the correlation coefficient which is classed as not significant means that there is at least one chance in ten that such a result could be obtained fortuitously from a sample of experimental results; a significant value is such that there is less than one chance in

twenty that it could arise fortuitously. Values intermediate between these are regarded as doubtfully significant, while values which could arise by chance less than once in a hundred times are considered very significant.

THE BIOS REQUIREMENTS OF SOME TOP-FERMENTATION BREWERY YEASTS IN RELATION TO THE NITROGEN SOURCE

By R. S. W. THORNE, PH.D.

(Received by the Chairman: 10/12/47)

It has been suggested that the bios requirements of yeast may vary to some extent according to the nature of the nitrogen source. In view of the undoubtedly complex nature of the synthetic reactions involved in the production of proteins by yeast from simple nitrogen compounds, such a suggestion is inherently likely.

Consequently it was felt that this question should be examined more closely since it is relevant to the propriety of assessing the relative nutrient values of different nitrogen sources under constant conditions of bios dosage.

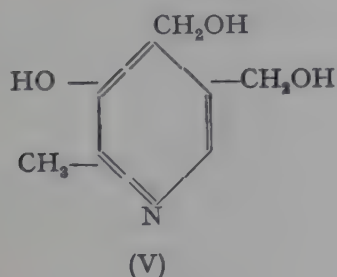
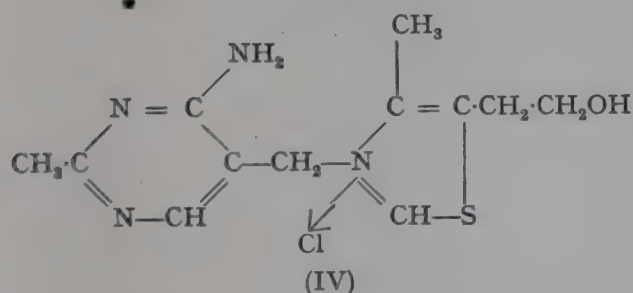
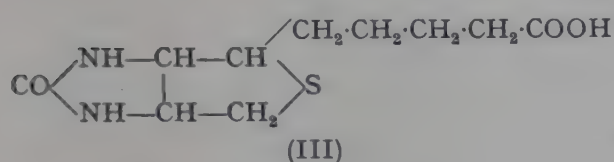
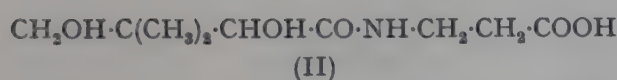
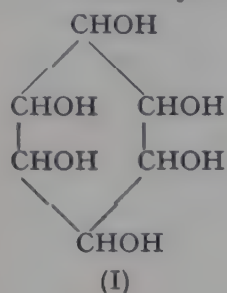
The experiments reported in the following paper show that certain differences in the bios requirements of yeasts are apparent when a comparison is made between ammonia and amino acids as nutrients. These differences are, however, quite small and do not affect to any appreciable extent the assessment of their relative nutrient values. No differences in the bios requirements of amino acids between themselves have been established. It has further been shown that the bios requirements of different top-fermentation brewery yeasts may vary quite markedly; in fact, out of four strains studied three different kinds of bios requirement were found.

Wildiers (*La Cellule*, 1901, 18, 313) discovered that yeast was unable to grow on a synthetic nutrient medium containing only sugar, ammonia and mineral salts, but that it would do so as soon as a small quantity of yeast-water was added to the medium. The unknown growth-promoting factor or factors evidently present in yeast-water was named "bios" by Wildiers. After four decades of research in the vitamin field it has become clear that Wildiers' bios is a complex of growth factors of which the most significant, from the point of view of yeast nutrition, are inositol, pantothenic acid, biotin, aneurin and pyridoxin. All these, and many other, growth factors are known or suspected to be

essential to the metabolic activities of living cells in general; but in the study of any particular organism, *e.g.* yeast, a growth factor only becomes nutritionally important when the cell is either incapable of synthesizing it, necessitating an external supply, or when the rate of synthesis is slow enough to act as a brake on the rate of growth.

The role of these growth factors in the economy of the cell is imperfectly understood at present. The function of inositol (I) is unknown; it differs from the other yeast growth factors in that its effect is only apparent in relatively large dosage. Pantothenic acid (II) and biotin (III) are nutritionally the most important of these factors.

Biotin is fully active in the exceedingly low concentration of 10^{-9} grms. *per* ml. It appears (Winzler, Burk and du Vigneaud, *Arch. Biochem.*, 1945, 5, 25) to take part in the synthesis of some nitrogenous substance involved in the fermentation cycle. The pyrophosphate of aneurin (IV) is the co-carboxylase of yeast which is involved in the pyruvic acid $\rightarrow$ acetaldehyde stage of the



fermentation cycle. Pyridoxal phosphate, a substance closely related to pyridoxin (V) functions as the coenzyme of amino acid decarboxylases (Baddiley and Gale, *Nature*, 1945, 155, 727) and also as the coenzyme of transaminase (Lichstein, Gunsalus and Umbreit, *J. Biol. Chem.*, 1945, 161, 311), so that it must clearly play an important part in protein metabolism.

Schultz, Atkin and Frey (*ibid.*, 1940, 135, 267) in a study of the bios requirements of yeast, found that when urea was used as a

nitrogen source, more bios IIB (biotin) and less bios IIA (pantothenic acid) were needed than when the nitrogen source was ammonia. They therefore suggested that the bios requirements of yeast might be considerably affected by the form of nitrogen supplied and consequently that such work as that of the present writer on the nutrient efficiency of different amino acids (this *Journ.*, 1941, 255) might profitably be re-examined with this consideration in mind.

The present paper reports the results of experiments on the bios requirements of four top-fermentation yeasts designated 6479 (*ibid.*, 1944, 222), G, W and Y in the presence of a number of different nitrogen sources, mostly amino acids. The yeasts are single cell cultures; G was originally isolated from a Burton yeast, W from a London yeast and Y from a Yorkshire yeast; the origin of 6479 is unknown but it closely resembles a Midland yeast.

EXPERIMENTAL

The basal nutriment medium used was as follows:—

- 1,000 mls. distilled water
- 2 grms. potassium dihydrogen phosphate
- 1 gm. magnesium sulphate (cryst.)
- 0.2 gm. calcium sulphate
- 0.01 gm. ferrous sulphate (cryst.)
- 60 grms. sucrose
- 3.5 mls. lactic acid
- 12 mls. 50 *per cent.* potassium lactate soln.

To this medium was added 0.35 gm. assimilable nitrogen in the form of ammonium phosphate or an amino acid, and the p_H of the medium adjusted to 5.0. Finally, appropriate amounts of the desired growth factors were added.

The media were put out in 100 ml. portions in special fermentation vessels (*ibid.*, 1939, 13), inoculated with washed suspensions of the yeasts and grown for 4 days with constant shaking at 21° C. Periodic measurements of yeast growth and fermentation were made in order to evaluate (a) the final yeast growth, (b) the initial relative growth rate and (c) the fermentation factor (*ibid.*, 1941, 255). The individual figures quoted in the subsequent tables are mean values obtained usually from

TABLE I

THE EFFECTS OF VARIOUS COMBINATIONS OF INOSITOL (I), PANTOTHENIC ACID (P) AND BIOTIN (B) USING YEASTS 6479, G, W AND Y, WITH AMMONIUM PHOSPHATE AS THE NITROGEN SOURCE

	No addition.	I.	P.	B.	IP.	IB.	PB.	IPB.
Final yeast growth in grms. per litre ± 0.4 .								
6479	1.1	1.2	4.7	3.4	5.5	5.5	9.7	11.9
G	3.2	3.5	2.9	5.1	3.5	9.0	5.5	7.8
W	3.4	4.1	4.8	2.7	5.0	4.2	7.0	8.7
Y	1.3	2.6	1.5	1.3	4.0	2.2	2.4	7.7
Initial relative growth rate ± 0.13 .								
6479	—	—	2.17	1.25	2.26	1.41	2.20	2.69
G	1.89	2.23	1.99	1.81	2.28	2.06	2.02	2.68
W	1.87	2.46	1.92	1.89	2.26	2.03	1.98	2.32
Y	2.04	2.28	—	—	2.62	2.41	1.86	2.98
Fermentation factor ± 0.02 .								
6479	0.37	0.42	0.71	0.51	0.72	0.62	0.89	(1.00)*
G	0.54	0.64	0.54	0.69	0.63	0.85	0.68	0.84
W	0.60	0.67	0.65	0.54	0.67	0.71	0.77	0.90
Y	0.45	0.62	0.43	0.46	0.66	0.64	0.54	0.88

* This is an arbitrarily chosen standard value of the fermentation factor.

TABLE II

THE EFFECTS OF VARIOUS COMBINATIONS OF INOSITOL (I), PANTOTHENIC ACID (P) AND BIOTIN (B) USING YEASTS 6479, G, W AND Y, WITH GLUTAMIC ACID AS THE NITROGEN SOURCE

	No addition.	I.	P.	B.	IP.	IB.	PB.	IPB.
Final yeast growth in grms. per litre ± 0.7 .								
6479	0.5	0.6	3.0	0.7	4.1	0.7	8.0	9.8
G	1.3	1.8	1.7	1.8	2.1	2.6	4.0	4.5
W	0.6	1.0	3.2	0.7	3.6	1.0	4.4	5.5
Y	0.4	0.5	1.3	0.5	3.7	0.8	2.2	5.4
Initial relative growth rate ± 0.28 .								
6479	1.42	1.68	2.82	1.86	2.32	1.26	1.95	2.28
G	1.59	1.60	1.59	1.49	1.79	1.51	1.76	1.99
W	1.49	1.19	2.05	1.09	2.17	1.27	2.32	1.98
Y	1.19	1.53	1.67	1.51	1.77	1.37	1.66	1.84
Fermentation factor ± 0.07 .								
6479	0.47	0.38	0.75	0.38	0.77	0.42	0.93	(1.00)
G	0.51	0.53	0.59	0.54	0.63	0.60	0.76	0.79
W	0.34	0.45	0.71	0.38	0.73	0.47	0.86	0.87
Y	0.39	0.51	0.61	0.44	0.69	0.47	0.71	0.84

measurements made on eight separate cultures. In all cases the standard deviations of these mean values are specified.

The first series of experiments was designed to ascertain the effects of inositol, pantothenic acid and biotin, used singly and in combination, upon the four yeasts when ammonium phosphate was the source of nitrogen. A considerable experience of the growth of yeast in synthetic media has shown that satisfactory growth is obtained if these growth factors are used in the following concentrations:—

Inositol: 20 mgrms. *per* litre;

Pantothenic acid (as calcium salt): 2 mgrms. *per* litre;

Biotin: 1 μ gm. *per* litre (1 μ gm. = 1/1000 mgrm.).

Accordingly, these concentrations were used in the present series of measurements. Eight media, differing in their bios content, were used, *viz.* no growth factors (control), inositol only, pantothenic acid only, biotin only, inositol + pantothenic acid, inositol + biotin, pantothenic acid + biotin, and inositol + pantothenic acid + biotin. The experimental results are given in Table I. The results of an exactly similar series of experiments using glutamic acid in place of ammonium phosphate as the nitrogen source are given in Table II, and the two sets of data may conveniently be considered together.

In the absence of all three growth factors none of the yeasts grows or ferments satisfactorily with either nitrogen source. The addition of inositol only or of biotin only produces as a rule only a small improvement if any; the addition of pantothenic acid alone tends to give a somewhat greater improvement. It is more informative to take the growth and fermentation in the presence of all three factors as a standard of comparison and to contrast with this the figures obtained when one or other of the factors is absent (the last four columns of Tables I and II). Since the figures for final growth, initial relative growth rate, and fermentation tend to run parallel with each other there is some justification in taking an average value derived from all three of them. This is done in Table IIA which shows the percentage reduction of this averaged growth characteristic caused by the omission of growth factors from the medium. The absence of biotin

reduces the response of all the yeasts, though the reduction is rather less marked with W and Y. It also seems that the amino acid is a little less sensitive to absence of biotin than ammonium phosphate. Absence of inositol causes only a slight reduction of response with yeasts 6479, G and W, but a distinctly greater one with yeast Y; the reductions are perhaps a little greater with ammonium phosphate than with glutamic acid. Absence of pantothenic acid produces a marked reduction in the growth of yeasts 6479, W and Y, but little or none in G; the need for pantothenic acid seems appreciably

TABLE IIA

PERCENTAGE REDUCTION IN GROWTH AND FERMENTATION OF YEASTS 6479, G, W AND Y IN THE ABSENCE OF DIFFERENT GROWTH FACTORS

Yeast.	Nitrogen source.	No inositol.	No pantothenic acid.	No biotin.
6479	Ammonium phosphate	16	48	37
G		26	0	35
W		16	31	27
Y		50	42	28
6479	Glutamic acid	14	75	37
G		10	26	31
W		4	60	19
Y		34	59	21

greater when glutamic acid is the nitrogen source than when this is ammonia. Apparently the differences among the yeasts in respect of bios requirements are more conspicuous than the differences between the nitrogen sources. Roughly, it can be said that all the yeasts require biotin, all but G require pantothenic acid and none but Y requires inositol. With glutamic acid the requirement for pantothenic acid is slightly greater, and that for inositol slightly less, than with ammonia.

The response of the four yeasts to variations in the concentrations of pantothenic acid and biotin was next examined, using ammonium phosphate as the nitrogen source. Inositol in a concentration of 20 mgrms. *per* litre was present in all the media. Then, with a fixed concentration of 1 μ gm. of biotin *per* litre, the concentration of pantothenic acid in different media was adjusted to 0.005, 0.026, 0.128, 0.64, 3.2 and 16

TABLE III

THE EFFECTS OF VARYING THE CONCENTRATIONS OF PANTOTHENIC ACID AND BIOTIN USING YEASTS 6479, G, W AND Y, WITH AMMONIUM PHOSPHATE AS THE NITROGEN SOURCE

	1 μ grm. biotin <i>per</i> litre.						2.0 mgrms. pantothenic acid <i>per</i> litre.			
	Pantothenic acid, mgrms. <i>per</i> litre:						Biotin, μ grms. <i>per</i> litre:			
	0.005	0.026	0.128	0.64	3.2	16	0.02	0.2	2	20
Final yeast growth in grms. <i>per</i> litre $\pm$ 0.4.										
6479	—	6.3	12.2	11.4	11.7	11.3	3.8	6.5	11.3	11.9
G	9.4	9.8	9.0	8.7	8.5	—	3.9	6.3	8.8	9.5
W	5.4	8.6	8.6	9.0	8.1	—	4.7	6.8	9.5	13.1
Y	—	3.1	7.5	7.5	7.4	—	3.5	6.2	8.6	—
Initial relative growth rate $\pm$ 0.08.										
6479	—	1.65	2.33	2.35	2.32	2.29	1.89	2.24	2.34	2.40
G	2.01	1.91	2.18	2.31	2.10	—	1.67	1.97	2.11	2.23
W	1.57	1.88	2.18	2.08	2.09	—	1.72	1.96	2.03	2.50
Y	—	2.32	2.50	2.47	2.63	—	2.40	2.49	2.47	—
Fermentation factor $\pm$ 0.02.										
6479	—	0.78	1.14	1.04	1.04	1.01	0.63	0.83	(1.00)	1.06
G	0.83	0.82	0.81	0.81	0.80	—	0.59	0.71	0.82	0.84
W	0.67	0.83	0.83	0.80	0.79	—	0.62	0.73	0.81	0.89
Y	—	0.63	0.75	0.73	0.73	—	0.56	0.71	0.75	—

TABLE IV

THE EFFECTS OF VARYING THE CONCENTRATIONS OF PANTOTHENIC AND BIOTIN, USING YEAST 6479, WITH AMMONIUM PHOSPHATE, ALANINE, LEUCINE AND GLUTAMIC ACID AS THE NITROGEN SOURCES

	1 μ grm. biotin <i>per</i> litre.						2.0 mgrms. pantothenic acid <i>per</i> litre			
	Pantothenic acid, mgrms. <i>per</i> litre:						Biotin, μ grms. <i>per</i> litre:			
	0.026	0.128	0.64	3.2	16		0.02	0.2	2	20
Final yeast growth in grms. <i>per</i> litre $\pm$ 0.5.										
Amm. phos. ..	7.7	14.9	14.0	14.4	13.9		4.7	7.9	13.9	14.7
Alanine ..	3.0	7.0	6.1	5.7	5.2		2.4	4.6	5.6	5.8
Leucine ..	2.8	9.1	8.9	6.5	6.5		3.4	6.0	8.4	7.1
Glut. acid ..	4.8	10.8	12.0	11.4	11.7		4.1	7.5	11.7	12.0
Initial relative growth rate $\pm$ 0.08.										
Amm. phos. ..	1.74	2.46	2.48	2.45	2.42		1.99	2.36	2.47	2.54
Alanine ..	1.22	1.50	1.51	1.50	1.55		1.56	1.54	1.55	1.46
Leucine ..	1.40	1.58	1.69	1.84	1.72		1.56	1.64	1.62	1.83
Glut. acid ..	1.75	2.04	2.14	2.03	1.98		1.95	2.06	2.05	2.10
Fermentation factor $\pm$ 0.02.										
Amm. phos. ..	0.77	1.13	1.03	1.03	(1.00)		0.62	0.82	0.99	1.05
Alanine ..	0.44	0.61	0.58	0.58	0.55		0.50	0.56	0.57	0.55
Leucine ..	0.50	0.71	0.71	0.68	0.67		0.49	0.61	0.69	0.70
Glut. acid ..	0.70	0.93	0.93	0.87	0.89		0.62	0.79	0.90	0.91

mgrams. *per* litre, *i.e.* the concentrations were successively increased by a factor of 5. Similarly, with a fixed concentration of 2 mgrams. of pantothenic acid *per* litre, the biotin concentrations were adjusted to 0.02, 0.2, 2 and 20 μ grams. *per* litre. The experimental data obtained by growing the four yeasts in these media are given in Table III. Inspection of these figures shows that for pantothenic acid there is with yeasts 6479, W and Y a maximal concentration at 0.128 mgrams. *per* litre; increasing the concentration beyond this value does not give any further improvement in the yeast response—indeed, there tends to be a just perceptible decline. Yeast G, as already found, does not require pantothenic acid and hence alterations in the concentration of this factor are without effect on its response. Over the range of concentrations of biotin examined there is a gradual improvement in the response of each of the yeasts which is, however, practically complete at 2 μ grams. *per* litre. To summarize, it can be said that varying the concentrations of pantothenic acid and biotin does not disclose any differences between the yeasts apart from the fact, already found, that yeast G does not need pantothenic acid.

An exactly similar series of experiments was carried out using only yeast 6479, but four different nitrogen sources, namely, ammonium phosphate, alanine, leucine and glutamic acid, with the results shown in Table IV. With ammonium phosphate, alanine and leucine, maximal response to pantothenic acid is obtained, as before, at a concentration of 0.128 mgrams. *per* litre; in the case of glutamic acid a slightly greater response is obtained when the concentration is increased to 0.64 mgrams. *per* litre. The response to biotin reaches a maximum value when its concentration is 2 μ grams. *per* litre. Thus the four nitrogen sources are alike in their quantitative requirements for pantothenic acid and biotin with the reservation that glutamic acid may possibly have a slightly greater requirement for the former. (This is not at variance with the previous finding that the yeasts are distinctly more sensitive to the *absence* of pantothenic acid when glutamic acid is the nitrogen source).

Attention was next directed to the effects of some other substances upon yeast growth and fermentation, namely, aneurin, pyridoxin, nicotinic acid and riboflavin. Yeast 6479

was employed, with ammonium phosphate as the nitrogen source. Inositol, pantothenic acid and biotin in the concentrations already specified, were used in all the media. The concentrations of the remaining factors were:—aneurin: 0.1 mgram. *per* litre; pyridoxin: 0.1 mgram. *per* litre; nicotinic acid: 0.1 mgram. *per* litre; riboflavin: 0.2 mgram. *per* litre. These were used singly and in all possible combinations, the results obtained for yeast growth and fermentation being shown in Table V. An analysis of these

TABLE V

THE EFFECTS OF ANEURIN (AN), PYRIDOXIN (PR), NICOTINIC ACID (NC) AND RIBOFLAVIN (RB) IN ALL POSSIBLE COMBINATIONS UPON YEAST 6479, WITH AMMONIUM PHOSPHATE AS THE NITROGEN SOURCE

	Final yeast growth in grms. <i>per</i> litre.	Initial relative growth rate.	Fermentation factor.
No addition ..	14.0	2.37	(1.00)
An	13.4	2.05	1.02
Pr	14.4	2.58	1.07
Nc	14.5	2.40	1.04
Rb	13.7	2.39	1.01
An + Pr	14.5	2.96	1.22
An + Nc	13.0	2.17	1.03
An + Rb	13.6	2.14	1.03
Pr + Nc	14.0	2.57	1.07
Pr + Rb	13.4	2.56	1.08
Nc + Rb	14.5	2.40	1.03
Pr + Nc + Rb ..	15.0	2.49	1.08
An + Nc + Rb ..	12.6	2.15	1.01
An + Pr + Rb ..	14.2	2.74	1.14
An + Pr + Nc ..	14.6	2.76	1.18
An + Pr + Nc + Rb	14.0	2.88	1.21
Standard deviations ..	± 0.4	± 0.06	± 0.02

figures enables the net result to be expressed very briefly. Aneurin alone has a slightly depressive effect, to the extent of 6 *per cent.*, on yeast response; pyridoxin alone has a slightly stimulative effect, to the extent of 4 *per cent.* Aneurin and pyridoxin in combination cause quite a noticeable improvement in response, the average value being 12 *per cent.* Nicotinic acid and riboflavin, singly or together, exercise practically no modifying effect on these results.

In order to ascertain the validity of this conclusion under a wider range of conditions a series of experiments was designed in which yeast 6479 was grown with ammonium phosphate, glycine, alanine, leucine, glutamic acid, aspartic acid, asparagine, arginine, histidine or tryptophane as the nitrogen source; inositol, pantothenic acid and biotin in the standard concentrations were used in all the media, together with

together cause a reduction of 3 *per cent.* in yeast response. Closer inspection of the data shows that the omission of aneurin and pyridoxin from the medium causes on the average a greater reduction in response when the nitrogen source is an amino acid than when it is ammonia: the average reduction with ammonia (from this and the previous experiment) is 10 *per cent.*, while the average reduction with amino acids is 18 *per cent.*

TABLE VI

THE EFFECTS OF ANEURIN (AN), PYRIDOXIN (PR), NICOTINIC ACID (NC) and RIBOFLAVIN (RB) UPON YEAST 6479, WITH AMMONIUM PHOSPHATE, GLYCINE, ALANINE, GLUTAMIC ACID, ASPARTIC ACID, ASPARAGINE, ARGININE, HISTIDINE AND TRYPTOPHANE AS THE NITROGEN SOURCES

	Ammonium phosphate.	Glycine.	Alanine.	Leucine.	Glutamic acid.	Aspartic acid.	Asparagine.	Arginine.	Histidine.	Tryptophane.
Final yeast growth in grms. <i>per</i> litre ± 0.4 .										
No addition ..	15.9	0.2	10.3	13.0	14.9	12.7	19.8	12.9	1.9	7.0
An ..	15.3	0.2	8.7	11.3	11.7	12.9	19.4	9.7	1.6	—
Pr ..	15.7	0.3	10.5	13.4	14.8	13.5	20.2	12.2	1.9	—
An + Pr ..	16.2	0.3	14.5	14.6	15.5	15.1	20.1	14.6	1.8	10.3
Nc + Rb ..	15.5	0.3	8.2	14.3	14.2	11.8	19.8	12.4	1.5	4.7
An + Pr + Nc + Rb ..	16.3	0.2	13.7	14.4	16.4	14.8	20.8	13.6	1.7	—
Initial relative growth rate ± 0.05 .										
No addition ..	2.48	0.70	1.61	1.76	1.79	1.61	2.49	2.09	0.82	1.20
An ..	2.18	0.58	1.59	1.84	1.90	1.80	2.44	1.62	0.93	—
Pr ..	2.65	0.61	2.03	1.86	1.98	2.04	2.59	2.42	0.94	—
An + Pr ..	2.95	0.51	2.39	2.02	2.32	2.43	2.89	2.52	0.93	1.37
Nc + Rb ..	2.52	0.64	1.45	1.55	1.87	1.51	2.34	2.06	0.93	1.18
An + Pr + Nc + Rb ..	2.90	0.69	2.25	1.93	2.10	2.30	2.80	2.57	0.96	—
Fermentation factor ± 0.01 .										
No addition ..	(1.00)	0.05	0.63	0.73	0.77	0.69	1.07	0.79	0.26	0.31
An ..	1.05	0.05	0.69	0.76	0.83	0.85	1.13	0.69	0.30	—
Pr ..	1.02	0.05	0.71	0.76	0.85	0.84	1.13	0.87	0.29	—
An + Pr ..	1.17	0.09	0.95	0.87	0.97	1.00	1.26	0.99	0.29	0.50
Nc + Rb ..	0.97	0.07	0.58	0.66	0.76	0.65	1.02	0.75	0.28	0.27
An + Pr + Nc + Rb ..	1.17	0.05	0.94	0.85	0.95	1.00	1.28	1.04	0.28	—

optional additions of aneurin, and pyridoxin singly or together, and nicotinic acid and riboflavin with and without aneurin and pyridoxin. The results of these experiments are summarized in Table VI. The average trend disclosed by an examination of these figures is that aneurin alone reduces the yeast response by 2 *per cent.*, pyridoxin increases it by 6 *per cent.* and aneurin and pyridoxin together by 16 *per cent.* Nicotinic acid and riboflavin

It should be observed that those amino acids, *e.g.* glycine and histidine, which, under the conditions of the writer's earlier experiments (*ibid.*, 1941, 255), were practically useless as yeast nutrients are not materially improved under the present conditions with known amounts of inositol, pantothenic acid, biotin, aneurin and riboflavin. It is instructive to compare the earlier results (1941) with these later ones (1947) using averaged growth

characteristics and taking the values for ammonium phosphate (100) as standards of comparison. The relevant figures are given in Table VIA. It will be seen that the agreement between the two sets of figures is

TABLE VIA

THE RELATIVE VALUES OF DIFFERENT NITROGEN SOURCES (AMMONIUM PHOSPHATE = 100) DETERMINED IN 1941 WITH INOSITOL, PANTOTHENIC ACID AND BIOS IIB, AND IN 1947 WITH INOSITOL, PANTOTHENIC ACID, BIOTIN, ANEURIN AND PYRIDOXIN

	1941.	1947.
Ammonium phosphate ..	100	100
Glycine	17	8
Alanine	55	57
Leucine	73	70
Glutamic acid	88	83
Aspartic acid	87	69
Asparagine	116	113
Arginine	76	78
Histidine	32	22
Tryptophane	51	33

remarkably close, considering the fluctuations in the response of yeast to a defined chemical medium which are always observed even over much shorter periods of time than 6 years. The only differences which might possibly be significant are found with aspartic acid and tryptophane, but the writer would hesitate to ascribe these to varying responses of the amino acids to the different bios supplements employed in the two sets of experiments.

DISCUSSION AND SUMMARY

The processes whereby a simple source of nitrogen such as an ammonium salt or an amino acid is converted by yeast into proteins must be of extreme complexity, involving the co-operation of many growth factors and the delicate balancing of many interlocking chemical reactions. The growth factors themselves will be the products of the yeast cells' own synthetic activity or, where this fails, will be assimilated directly from the nutrient medium. It seems *a priori* rather probable that, since different nitrogen sources will alter at any rate the initial step of the protein synthesis, they may also necessitate a somewhat different balance of the growth factors to achieve the maximum yeast response of which they are capable. On the other hand, it can hardly be doubted

that this initial stage of the nitrogen metabolism is but a small proportion of the whole series of transformations taking place, and that the requirement for those growth factors not implicated in it should be unaffected by the nature of the nitrogen source.

The experimental results presented in this paper do indicate that there are differences, though not very great ones, between the sensitivities of yeasts to bios deficiencies when the source of nitrogen is, on the one hand, ammonia, and on the other, amino acids. Perhaps the most significant difference of this kind is that which is shown when aneurin and pyridoxin are absent from the media. The growth of yeast is lowered by about 10 *per cent.* as a result of this deficiency when the nitrogen source is ammonia, but is lowered nearly twice as much if the nitrogen source is an amino acid. Since pyridoxin, as mentioned above, appears to be directly concerned in the decarboxylation and transamination of amino acids this result, as far as it goes, fits into the picture quite well. On the data at present available it is not possible to say whether there are any differences among the amino acids themselves in their requirements for aneurin and pyridoxin. A further conclusion from the results presented is that absence of biotin causes a differential response as between ammonia and amino acids; yeast seems more able to dispense with this factor when the nitrogen source is an amino acid.

The differences in bios requirements between the nitrogen sources examined are, however, much less conspicuous than the specific differences between the four yeasts examined even though each of these is of the top-fermentation brewery type. To obtain adequate growth and fermentation under the experimental conditions adopted, irrespectively of the nitrogen source, yeast G requires biotin, yeasts 6479 and W require pantothenic acid and biotin, and yeast Y requires inositol, pantothenic acid and biotin. As far as the quantitative requirements for pantothenic acid and biotin are concerned, maximal yeast response is obtained when the concentrations of these factors are approximately 0.1 mgrm. *per* litre and 2 μ grms. *per* litre respectively; these values appear scarcely to be affected by the nature of the nitrogen source.

Finally, such differences as may exist between the bios requirements imposed by

different nitrogen sources are evidently so small as not to invalidate comparative studies of the nutrient efficiencies of different amino acids made with constant dosages of the growth factors.

The author's best thanks are due to Miss

Doreen L. Birkett and Miss Myra G. Hulse for assistance with the experimental work.

Institute of Brewing Research Laboratories,
University of Birmingham.

8th December, 1947.

A STUDY OF ROPINESS IN BEER

By J. L. SHIMWELL, D.Sc., F.R.I.C.

Part III. Ropiness due to *Lactobacilli*

In Parts I and II of this Study (this *Journ.*, 1947, 280; 1948, 237) the incidence of ropiness in beer due to *Acetobacter* species and tetrad-forming *Streptococcus* species respectively has been discussed. Which of the two types is the more prevalent it is difficult to estimate. Most brewers would probably say the latter, since cocci are widely believed to be the chief cause of ropiness. It has to be borne in mind, however, that both *Acetobacter viscosum* and *A. capsulatum* often adopt an almost spherical form (*loc. cit.*), and several cases of ropiness submitted to the writer as being due to cocci have proved to be due to one or other of these *Acetobacter* species.

As regards virulence, there is little doubt that ropiness due to *Str. damnosus* varieties is, of the two types, far more dangerous and difficult to combat, for exclusion of air cannot be used to suppress growth, as is the case with the acetic acid bacteria.

Whatever may be the *relative* prevalence of ropiness due to these two types of bacteria the writer's experience is that together they are responsible for most instances of ropiness occurring in British beers. Out of all the cases encountered in the last twelve years only two were due to other bacterial types, namely *lactobacilli*. It is felt, however, that this study would not be complete without reference to such rare cases.

In the first of these instances, previously referred to (Shimwell, *ibid.*, 1936, 127), the organism was not actually isolated from ropy beer, but from a sample of pitching yeast. On inoculating unhopped beer with a pure culture of the organism only acidity and "silky" turbidity was produced, but when hopped beer was substituted bacterial

growth took place but ropiness was produced. The hypothesis was advanced that possibly the hop antiseptic present might have stimulated the bacterial cell to surround itself with slime, a phenomenon somewhat analogous to that later observed in the case of *Str. damnosus* var. *viscosus*, where CO₂ saturation appeared to fulfil a similar function (Part II of this study, *ibid.*, 1948, 237).

Unfortunately, cultures of this organism were not preserved and it has therefore not been possible to make a proper study of it. Since, however, it was Gram-positive, non-motile, facultative and aciduric, it seems probable that it was a species of *Lactobacillus*.

Compared with *L. pastorianus* it was a much smaller and shorter rod about $0.4\mu \times 1.5\mu$ (although the variable morphology of the former species must be borne in mind). When producing ropiness in hopped beer the organism grew chiefly as single rods with occasionally pairs (Fig. 1), whilst in unhopped beer chains were the predominating form (Fig. 2). (The high magnification ($\times 1200$) should be noted.) The cultural characters on solid media were uncharacteristic, similar to those of other *lactobacilli*.

Lactobacillus pastorianus var. *brownii*.—The other slime-producing *lactobacillus* was isolated by B. M. Brown from ropy bottled ale and was handed to the writer for study.

On inoculating beer with this organism, the same phenomenon was encountered as with *Str. damnosus* var. *viscosus* (this study, Part II, *loc. cit.*), i.e. ropiness was not produced in "flat" beer, only acidity and turbidity ensuing, but if bottled beer saturated



FIG. 1



FIG. 2

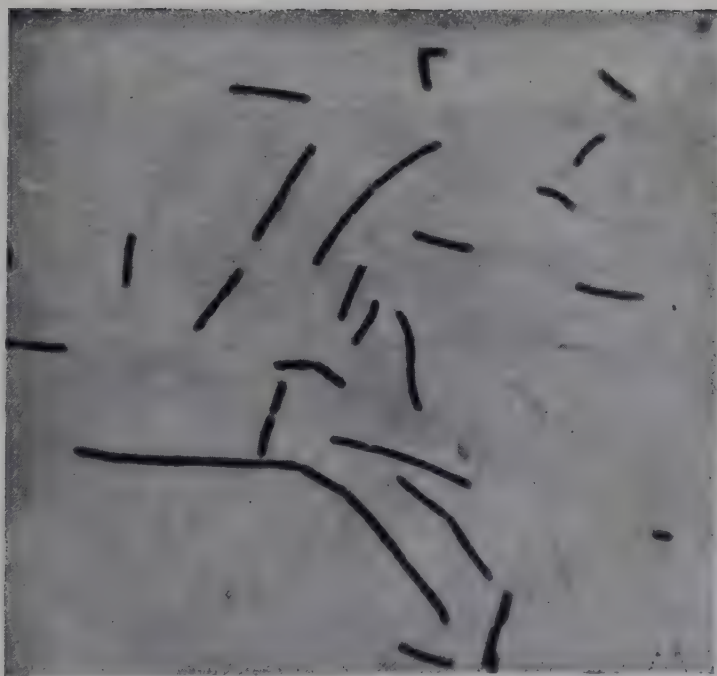


FIG. 3

L. pastorianus var. *brownii* $\times 1000$ —Gram stain



FIG. 4

L. pastorianus. Streak culture $\times 100$

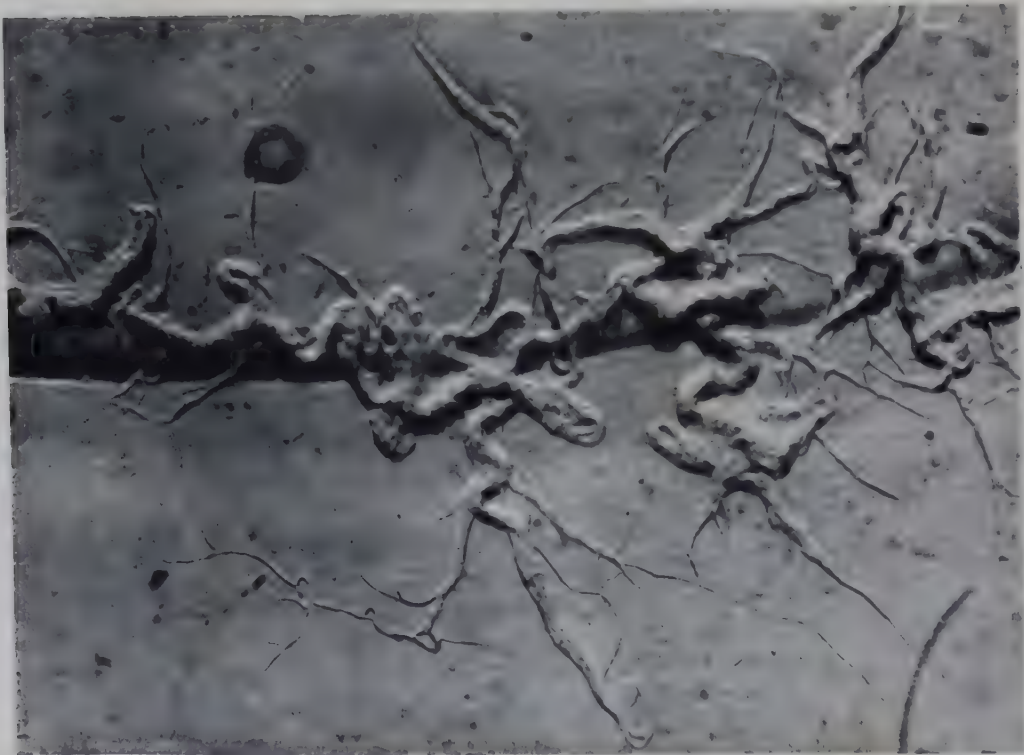


FIG 5

L. pastorianus var. *brownii*. Streak culture $\times 100$

under pressure with CO₂ was used, then a lesser degree of turbidity occurred but the liquid became highly ropy. This was in accordance with the original observation made by Brown that the beer from which the bacterium was isolated had an abnormally high gas content.

Whilst awaiting an opportunity to make a systematic study of this organism, it was maintained as a stab culture, firstly in unhopped wort agar and afterwards in hopped beer gelatin. Whether this has been responsible for a change in habit is not certain, but when a study of the mechanism of slime production was recently undertaken it was found that, as in the case of *Str. damnosus* var. *viscosus*, all ability to produce ropiness in beer had been lost. Growth in highly carbonated pasteurized beer was restricted as before, but the previous accompanying viscosity could not be induced. The organism has been inoculated into beers of various types, including lager beer, with and without the additions of glucose, fructose, maltose, sucrose and dextrin, and has been grown under varying conditions of anaerobiosis, alcohol concentrations, p_H , and temperature, all without effect. In all cases merely various degrees of acidity and off-flavour, accompanied by the "silky" turbidity characteristic of the presence of rod-shaped bacteria, have been the sole type of spoilage produced.

Disappointing as was this loss of ropiness, it was not without interest, for during the somewhat lengthy experiments briefly referred to above, it was difficult to distinguish (technologically at any rate) between the organism under study and the well-known *Lactobacillus pastorianus* itself. Since the latter species has never been previously recorded as producing ropiness, it was thought well worth while to proceed with the study of its distinguishing characters in order to decide whether it could be regarded as a strain of *L. pastorianus* or, if not, to record such distinguishing characters in case a similar organism should be subsequently encountered by other workers.

Morphology and Cultural Characters.—In its morphology the organism is similar to *L. pastorianus*, although, perhaps, the rods may be usually (but not invariably) somewhat thicker (0.9 μ to 1.2 μ). The same variability in length is exhibited as in the case of

L. pastorianus, rods of 3 μ to 200 μ or more being encountered. On solid media very short rods are similarly often produced. Fig. 3 shows rods from a beer culture, Gram stained.

The cultural characters on solid media are also similar to those of *L. pastorianus*, surface colonies on beer gelatin being irregularly circular, often with rhizoid processes, stab cultures in the same medium beaded to filiform (sometimes arborescent if a low gelatin concentration is employed to give a soft jelly). Stab cultures on wort agar are similar but with thicker growth. On wort agar slants a greyish white beaded uncharacteristic growth develops.

The surface growth on wort gelatin further illustrates the resemblance between this organism and *L. pastorianus*, both organisms producing a rather characteristic rhizoid streak which may have some value in distinguishing them from other brewery lactobacilli (Figs. 4 and 5).

In liquid media some divergence from *L. pastorianus* is exhibited, for whilst both organisms grow better in wort than in yeast extract plus glucose, the one under study showed a greater preference for wort, growth in yeast extract glucose being relatively poor. A similar phenomenon has been recorded by Henneberg for *L. lindneri* (*Bacillus lindneri*, Henneberg, *Handbuch der Gärungsbakteriologie*, Berlin, 1926). As will be seen later, the present organism exhibits other resemblances to *L. lindneri*.

In animal media, *viz.* glucose broth, glucose peptone, and litmus milk no growth was obtained, the organism, like *L. pastorianus*, evidently requiring the vegetable growth factors present in yeast extract and more particularly in malt wort.

Physiology.—*L. pastorianus* belongs to the heterofermentative lactic acid bacteria, producing in addition to lactic acid considerable quantities of volatile acid, together with carbon dioxide. The present organism was also found to be heterofermentative, in a wort culture approximately 30 per cent. of the total acid produced being volatile. (It is interesting to note, in this connection, that the beer streptococci, notably *Str. damnosus* and its varieties, are homofermentative, the acid produced being almost entirely lactic).

To illustrate what are considered the close relationships between the present organism,

	<i>L. pastorianus</i>	<i>L. lindneri</i>	<i>L. pastorianus</i> var. <i>brownii</i> .
Optimum temperature	30° C.	19° C. (Henneberg)	30° C.
Maximum temperature	38° C.	38° C. (Henneberg)	38° C.
Growth in wort	Good	> Yeast extract	> Yeast extract
Growth in yeast-extract-glucose	Good	< wort	< wort
" " glucose broth	Nil	Nil	Nil
" " litmus milk	Slight	Nil	Nil
<i>Sugar Fermentations.</i>			
Glucose	+	+	+
Fructose	+	-	-
Galactose	+	-	- (?)
Sucrose	+	-	-
Maltose	+	+	+
Lactose	±	-	-

L. pastorianus and *L. lindneri*, other physiological characters are compared and contrasted in the accompanying table.

It is particularly interesting to note that the sugar fermentations (in yeast extract) of the present organism are the same as those of *L. lindneri* and notably different from those of *L. pastorianus*, whilst in its temperature relations the reverse is the case. (The sugar fermentations of *L. lindneri* are those recorded by Henneberg (*Z. für Spiritusind.*, 1903, 23, 243)).

Since Brown's organism, whilst attacking only glucose and maltose (of the brewery sugars) possesses the temperature relations, cultural characters and heterofermentative nature of *L. pastorianus*, it seems desirable to regard it as a variety of that species and the name *L. pastorianus* var. *brownii* is therefore proposed. For technological purposes it can be referred to as *L. brownii* without implying species rank as has been suggested in the case of *Str. limosus* in Part II of this study (*loc. cit.*).

The Predisposition of Beer to Ropiness by L. brownii.—When considering the technological significance of the sugar "fermentations" of an acid-producing organism such as the present one, it must be remembered that acid production, although the conventional criterion, is not necessarily the only one of importance. It has been shown, for example, in Part I of this study (*loc. cit.*) that *Acetobacter capsulatum* and *A. viscosum* do not produce acid from dextrin and maltodextrin, yet those substances are the only

ones from which they can fabricate ropiness.

This point has, therefore, been particularly borne in mind in the case of *L. brownii*. It has been found, however, that not only (of the brewery sugars tested) were glucose and maltose the only sugars producing acid; they were also the only ones encouraging enhanced growth of the bacterium. In the case of fructose, sucrose, lactose and dextrin no more growth occurred than in plain yeast extract. It seems reasonable to suppose, therefore, that the presence of glucose and/or maltose is the probable predisposing factor for the production of ropiness. In addition to this it would appear that a high concentration of CO₂ is also necessary. This is in accordance with the original habitat from which the organism was isolated—namely a primed, carbonated, bottled mild ale.

Although sucrose was not attacked under the conditions of the experiment, it must be remembered that under actual brewery conditions cane sugar primings are, in beer, subject to the action of invertase derived from the yeast, so that priming either with invert or cane sugar would potentially predispose the beer to ropiness if *L. brownii* were present. Residual maltose, due to too sugary a wort and/or to incomplete attenuation would potentially exercise a similar effect. The growth phase of the bacteria at the time of priming would probably be an important factor, however, as has already been pointed out in the case of *Str. limosus* (*loc. cit.*).

Mechanism of Slime Production.—As in the case of *A. capsulatum*, *A. viscosum* and *Str. limosus* the production of ropiness by *L.*

brownii has again been found to be due to the formation of gelatinous capsules surrounding the cells. Before the organism under study had lost its slime producing propensity, capsule staining was carried out on cells from rosy beer media, positive staining with Plimmer and Paines flagella stain showing

voluminous slimy material surrounding the rods. No photomicrograph is, however, available, but the microscopic picture obtained was similar to that of *Str. limosus* accompanying Part II of this study (*loc. cit.*) except, of course, that the cells were rods and not cocci.

THIRTY-FIRST REPORT ON THE TRIAL OF NEW VARIETIES OF HOPS, 1947

By PROF. E. S. SALMON

A LARGE number of new varieties of hops (originally raised in the Experimental Hop-garden at Wye College) are now sufficiently established at East Malling Research Station for their distinctive characters to be ascertained. The results obtained for the season of 1947 are given in the present Report.*

The measuring of the crop was carried out by Mr. F. H. Beard. The total growth of the hops was dried by Mr. W. Dence, in the oast-house fitted with the Sirocco hot-air system.

The information given below is grouped under the following headings:—

- (1) Origin of the new varieties.
- (2) Actual and estimated yields.
- (3) Number of bushels required to the cwt.
- (4) The resins-contents.
- (5) The crop of new varieties marketed by registered growers.
- (6) New varieties resistant to *Verticillium* wilt.
- (7) Machine-picking of hops.
- (8) Brewing trials with new varieties of hops.
- (9) Brewing trials with new varieties of the 1945 and 1946 crops.
- (10) The challenge cups for new (Wye) varieties of hops.
- (11) Summary.

* Copies of previous Reports can be obtained on application to the Secretary, Research Station, East Malling, or to the Secretary, Wye College, Kent.

I. ORIGIN OF THE NEW VARIETIES

"Brewer's Gold" (C9a), "Bullion Hop" (Q43), OW76, OO21, FF8, OW94, R3/100, 287a, 334a, R3/23a.—Seedlings raised from a wild hop from Manitoba, Canada (BB1).

"Pride of Kent" (170a), OB9, II149, ADD4, AEE55, OT9, AOP2, X92a, X113, 342, AAA3, AEE36.—Seedlings of "Brewer's Gold" (C9a) (see above).

OH20.—Seedling of II149 (see above).

OT156, OG13.—Seedlings raised by "crossing" "Brewer's Gold" (C9a) (see above) with a male hop obtained from Oregon, U.S.A. (OO63).

AOO1, AOO25, 143.—Seedlings of OI59 ("Brewer's Gold" (C9a) × male hop from Oregon).

AOM47.—Seedling of OR137 ("Brewer's Gold" (C9a) × male hop from Oregon).

OD18, OR38.—Seedlings of R3/100 (see above).

"Nonsuch Hop" (OB53), FF60.—Seedlings of 334a (see above).

OW3.—Seedling raised by "crossing" "Nonsuch Hop" (OB53) with a male seedling (341) of *Humulus americanus* var. *neomexicanus*.

OM26.—A clone-plant, and bud sport, of "Nonsuch Hop" (OB53) (see above).

AOO13.—Seedling of FF60 (see above).

OS141.—Seedling of 411 (seedling of BB1 (wild hop from Manitoba)).

Y90.—Seedling raised from the American wild hop (*Humulus americanus* var. *neomexicanus*).

"College Cluster" (NI5bis), OR55.—Seedlings of Y90 (see above).

AOP6, OG3, OJ47.—Seedlings of EE92 (seedling of Y90 (see above)).

OF106, AOS6, AOS31, AOR10, AOQ8, AOQ12, R4/101a, AOT54.—Seedlings of R1/94 (seedling of Y90 (see above)).

R1/37.—Seedling of AOS14 (seedling of R1/94 (see above)).

"Malling Midseason" (BB28), "Fillpocket" (Z62), EE77, V32, OP13.—Seedlings of M45 ("Oregon Cluster" × English male) × English male hop.

"Quality Hop" (OO63), CC77, L11, R1/94a, Z74, ON78, R4/94a.—Seedlings of OP13 (see above).

OK40.—Seedling of R1/94a (see above).

ADD50, ACC5.—Seedlings of L11 (see above).

42a.—Seedling of OP79 (seedling of M45 (see above)).

HH50.—Seedling of OQ17 (seedling of M45 (see above)).

DD97, EE36.—Seedling of OJ93 (M45 (see above) × W78 (male seedling of var. *neomexicanus*)).

OF8.—Seedling of S41 (M45 (see above) × W78 (see above)).

"Brewer's Favourite" (OP21).—Seedling of K52 ("Oregon Cluster" × English male hop) × English male hop.

Z13, R1/55, 401.—Seedlings of "Brewer's Favourite" (OP21) (see above).

K31.—Seedling of Z100 ("Golden Hop" × OH2 (male hop from Oregon)).

"Concord Hop" (OH44).—Seedling of 309 ("Golden Hop" × English male hop) × English male hop.

"Brewer's Stand-by" (HH44), "Sunshine Hop" (V94), OV59, EE64, OT22.—Seedlings of OS99 (seedling of B20, a "Golding" hop of unknown parentage).

"Early Promise" (X35).—Seedling raised by "crossing" an English hop (D9) of unknown origin with a selected English male hop (B1).

"Early Choice" (FF21), AA102.—Seedlings of "Bramling."

AA101, DD12.—"Bramling" × R4/25a (male seedling of *H. americanus* var. *neomexicanus*).

OW33, OW92, R4/80a, K1.—"Bramling" × OL45 (male seedling of wild hop from Manitoba, Canada (BB1)).

OH1.—"Early Bird" × F17 (male hop from Oregon).

OT91.—Seedling of OV7 ("Late Bavarian" × G13 (English male hop)).

"John Ford Hop" (WFA90), "Northern Brewer" (WFB135), WFA12, clone-plant of

WFB135, "Wye Field Golding" (WFC81).—Seedlings raised by "crossing" "Canterbury Golding" with the male seedling OB21 ("Brewer's Gold" (C9a) × OY1, a male hop from California).

OO81, OR47.—Seedlings of Fuggle.

2. ACTUAL AND ESTIMATED YIELDS.

For the purpose of measuring the number of bushels of hops picked from each variety, the Imperial bushel measure is used. The hops are weighed when dried on the kiln. From the actual weight of a known number of bushels (Imperial) from a known number of hills, calculation can be made as to (1) the number of bushels (Imperial) of green hops required to the cwt. of dried hops; and (2) the yield per acre.

The season of 1947 was unfavourable in many districts for the growing of a full crop of healthy hops, and at East Malling Research Station the conditions were such that crop records of a large number of the varieties could not be obtained. Of the 84 new varieties, of which crop records were obtained (see Table I), 2 cropped at the rate of over 27 *cwt.* to the acre, and 13 at from 20 *cwt.* to 24½ *cwt.*

Mr. F. H. Beard has supplied the following comments, together with the rainfall figures at East Malling: "The severe weather in January and February, followed by a very wet March, delayed winter and spring work in the gardens, and cutting was not finished until mid-April. May and June were slightly wetter, while July was considerably wetter, than normal. These conditions favoured the spread of Downy Mildew, lateral spikes at the end of July being very numerous on many varieties. Until the end of August the hops were in excellent condition, but then the absence of any useful rain during August was having effect and patches of red spider damage were appearing. The September rain did not come until the 17th, too late to be of any benefit. A large number of varieties could only be "picked over," while some were not picked at all, and crop records for 81 varieties (including "Brewer's Gold" (C9a), "Bullion Hop" (Q43), "Nonsuch Hop" (OB53) and OM26) were not available. The chief reason for this was the incidence of red spider; if the weather had been consistently dry, there would, I think, have been less trouble from red spider! The relatively damp conditions until August did not

TABLE I (Abridged)**

Ref. No. or name of variety.	No. of hills picked.	No. of imperial bushels picked.	Average No. of imperial bushels per hill.	Total weight of dried hops (lb.).	No of imperial bushels of green hops to cut. dried hops.	Estimated yield per acre (889 hills to acre).
EARLY VARIETIES						
OJ47	10	48	3.00	55	98	27½
AQ86	15	41½	2.77	43	108	22½
"Early Promise" (X35)	15	34	2.27	36	106	19
"Early Choice" (FF21)	16	36	2.25	36	112	17½
OT22	16	37½	2.34	32	131	16
ACC5	15	31	2.07	30	116	16
AA102	15	30	2.00	29	116	15½
MIDSEASON VARIETIES						
"College Cluster" (N156is)	†16	46	2.88	48	107	27½
OG8	16	53	3.31	48	124	23½
*OW76	15	35½	2.37	43	92	22½
"Brewer's Stand-by" (HH44)	15	38½	2.57	42	103	22½
AQO13	15	35½	2.37	41	97	21½
EE64	15	35	2.33	38	103	20
AQO12	15	30½	2.03	37	92	19½
WFA12 (a clone-plant of WFB135 "Northern Brewer")	16	32½	2.03	38	96	18½
OV59	16	34	2.12	38	100	18½
OW92	16	31½	1.97	36	98	17½
R1.37	16	35½	2.22	36	110	17½
"Malling Midseason" (BB28)	16	26	1.63	36	81	17½
HH50	15	31½	2.10	33	107	17½
DD12	16	34	2.12	35	109	17½
"Wye Field Golding" (WFC81)	16	28½	1.78	35	91	17½
OH1	16	33	2.06	34	109	16½
OD18	14	28½	2.04	29	110	16½
AOP6	15	24½	1.63	30	91	15½
OP106	16	37	2.31	32	130	15½
DD97	12	19	1.58	24	89	15½
AA101	16	30½	1.91	32	107	15½
OK47	16	29½	1.78	32	100	15½
OT9	16	26	1.63	32	91	15½
R4.90a	16	30	1.88	31	108	15½
42a	9	15	1.67	17	99	15
"John Ford Hop" (WFA90)	16	18½	1.16	24	86	12
LATE VARIETIES						
OG13	15	37½	2.50	47	89	24½
AOR10	15	42½	2.83	46	103	24½
OR38	15	38½	2.57	43	100	22½
OR55	15	35	2.33	42	93	22½
K31	15	33	2.20	41	90	21½
OB9	12	24	2.00	31	87	20½
*OO21	15	33	2.20	38	97	20
"Sunshine Hop" (V94)	16	36	2.25	40	101	19½
"Concord Hop" (OH44)	16	37½	2.08	38	111	19½
ADD50	†13	37½	2.17	36	101	19
K1	15	32½	2.17	35	130	18
V32	15	40½	1.31	35	110	17½
*OW94	16	35½	2.22	36	98	17½
*OF8	16	31½	1.97	36	123	17½
OK40	16	39½	2.47	36	115	17½
EE77	15	34	2.27	33	115	17½
OS141	16	35	2.19	35	112	17½
AOS31	16	35	2.19	35	112	17½
ADD4	14	27	1.93	30	101	17
AOQ8	14	31½	2.25	29	122	16½
"Brewer's Favourite" (OP21)	16	33	2.06	33	112	16½
X113	403	707	1.75	824	96	16½
AOM47	15	27	1.80	30	101	15½
OT156	15	18½	1.23	30	69	15½
AEE36	15	28	1.75	32	98	15½
L11	16	32½	2.17	29	126	15½
R4/101a	15	27	1.80	29	104	15½
*R3/23a	15	20½	1.46	27	85	15½
CC77	14	27½	1.96	27	114	15½
OT91	14	28½	1.78	30	106	15
	16	24½	1.53	30	91	15

* Grown with only one bine to the string.

† 1,037 hills to acre.

‡ 1,778 hills to acre.

** The full table will be found in the Report as issued from the Research Station, East Malling, Kent.

encourage the spread of red spider, but did favour the spread of Downy Mildew, so that our spray programme was concentrated on the control of the latter trouble. The very hot and dry weather in August caused a rapid increase in red spider at a time when control was difficult. Quite apart from red spider damage most varieties showed signs of feeling the drought by loss of large leaves and generally wilted appearance. By the second week of September even the very late varieties were fully ready for picking. If we had been able to start picking a week earlier we should have completely picked many more varieties."

		Inches.	30 years' mean Inches.
1946	October ..	1.84	2.82
	November ..	4.88	3.15
	December ..	2.29	2.65
1947	January ..	2.17	2.53
	February ..	1.35	1.82
	March ..	4.97	1.52
	April ..	1.54	1.99
	May ..	2.06	1.77
	June ..	2.17	1.44
	July ..	3.69	2.20
	August ..	0.05	2.27
	September ..	1.53	2.08

Of outstanding interest among the early varieties is OJ47, which gave the phenomenal yield, for an early variety, of $27\frac{1}{4}$ *cwt.* to the acre.* As pointed out below, OJ47 is one of the varieties which has been discovered by Dr. W. G. Keyworth to be resistant to *Verticillium* wilt. Its crop-records during its trials at East Malling are as follows:—

Year.	No. of imperial bushels of green hops to <i>cwt.</i> dried hops.	Estimated yield <i>per acre.</i>
1940	94	$18\frac{1}{2}$
1941	80	$18\frac{1}{2}$
1942	92	$21\frac{1}{2}$
1943	90	$21\frac{1}{2}$
1944	95	$23\frac{1}{2}$
1945†	—	—
1946	99	$10\frac{1}{2}$
1947	98	$27\frac{1}{2}$

Attention may be drawn also to "Early Choice" (FF21), which yielded at the rate of $17\frac{3}{4}$ *cwt.* to the acre. This variety has recently been described and illustrated in a

* In the *Reports* for 1940 to 1946 this variety has been classified as Midseason; its behaviour in recent years, however, has shown that it is early in season.

† Crop not recorded owing to attacks of "mould."

leaflet issued from Wye College.* It is regular in cropping, developing its cones well, even in cold, wet, comparatively sunless seasons, as in 1946, with yields of $17\frac{3}{4}$ to 20 *cwt. per acre.* The densely-clustered cones stand well away from the leaves and are firm and easy to pick. It is considered by judges to have a "Golding" aroma and has been found to keep well in storage; the preservative value is higher than that generally found in "Bramblings." A valuable character is its immunity from the effects of Mosaic Disease, whereas, as is well known, the "Bramling" is liable to be severely affected by this disease. During its 19 years under observation at Wye, "Early Choice" has proved very resistant to Downy Mildew and also resistant to "mould," and Mr. F. H. Beard has reported the same behaviour at East Malling. "Early Choice" has been planted up by four hop-growers in Kent and by five in Worcestershire.

Notable among the midseason varieties for a heavy yield unaffected by the dry season is "College Cluster" (N15bis), with $27\frac{3}{4}$ *cwt.* to the acre, although possibly its lower P.V. of 54, as against a P.V. of 66 in 1946, may have been caused by unfavourable growing conditions. Among the newer midseason varieties mentioned in the last *Report* is OW76, of the same parentage as "Brewer's Gold" and "Bullion Hop"; this in 1947, grown with only one bine to the string, cropped at the rate of $22\frac{3}{4}$ *cwt.* to the acre, with a P.V. of 74; it appears, therefore, that this variety withstands semi-drought conditions. It is interesting to find also that under the same conditions "Brewer's Standby" and "Northern Brewer," with a P.V. of 105 and 86, respectively, gave yields of $22\frac{1}{4}$ *cwt.* and $18\frac{3}{4}$ *cwt.*

Among the late varieties, OB9 (a seedling of "Brewer's Gold") gave in the dry season of 1947 a yield of $20\frac{1}{2}$ *cwt.*, and a P.V. of 76; this variety well deserves a brewing trial. "Sunshine Hop" (V94) cropped at the rate of $19\frac{3}{4}$ *cwt.*, and gave a P.V. of 75. The variety OH44 gave a yield of $19\frac{1}{2}$ *cwt.*; in a leaflet recently issued from Wye College* this hop has been described and figured and given the name of "Concord Hop." Its bold cones somewhat suggest the Fuggle, but in

* E. S. Salmon: "Two New Hops, 'Early Choice' and 'Concord Hop'." Leaflet, Wye College, Kent. January, 1948. Price 2s. 6d.

other respects it clearly belongs to the "Golding Variety" class; the cones, however, have more "petals" and show richer condition. The cones develop well, "hang" well and are easy to pick. During its 24 years under observation at Wye it has proved to be resistant to Downy Mildew and "mould," and at East Malling Mr. F. H. Beard has found the same to be the case. For 16 consecutive years at East Malling it has, with two exceptions, yielded from 20 to 26 *cwt.* per acre. The excellent crop record and richness in soft resins make this a valuable variety for both brewer and grower. It has been planted up by three growers in Kent, two in Worcestershire and one in Sussex, and will be planted this year by a brewer-grower.

Of special interest among the late varieties is OR55, with a yield of 22½ *cwt.* and a P.V. of 82. This variety, like OJ47 among the early hops, has been found by Dr. W. G. Keyworth to be resistant to *Verticillium* wilt (see below). Its crop records during its trials at East Malling are as follows:—

Year.	No. of imperial bushels of green hops to <i>cwt.</i> dried hops.	Estimated yield per acre.
1940	82	18
1941	109	19½
1942	80	22½
1943	83	25½
1944	107	18
1945*	—	—
1946	85	17½
1947	93	22½

3. NUMBER OF BUSHELS REQUIRED TO THE *CWT.*

Among the early varieties, the heaviest weighing was OJ47, with 98 bushels to the *cwt.*, and the lightest weighing, OT22 with 131 bushels. Of the midseason varieties, OH20 was the heaviest, with 77 bushels, and OF106 the lightest, with 130 bushels. Of the late varieties, both AOM47 and AA3 required only 69 bushels to the *cwt.*, while K1 took 130 bushels.

4. THE RESINS-CONTENTS.

The analyses were carried out at Wye College, by Messrs. E. G. Bousfield, and G. A. Thomas, directed by Dr. A. H. Burgess, under a grant from the Institute of Brewing. The samples were stored in bottles at room temperature until analyzed. The analyses,

* Crop not recorded owing to attacks of "mould."

made during October and November, 1947, are given in Table II.

In the early varieties both OT22 and OJ47 have high preservative values; OJ47 is a heavy cropper, but it remains to be seen whether OT22, with a yield of 16 *cwt.*, will prove of commercial value.

Of the midseason varieties, "Brewer's Stand-by" is easily first, with a P.V. of 105, being nearly as rich as "Brewer's Gold." This variety deserves wider cultivation; it appears to be resistant to Downy Mildew as regards the cones. "John Ford" comes second, with a P.V. of 98, and "Northern Brewer" third, with 86. "John Ford" gave only 12 *cwt.* to the acre, due to not yet being fully established; "Northern Brewer" gave 18¾ *cwt.*; it is satisfactory to find that the latter hop is being planted up in Kent and in Worcestershire, and sets are being asked for from abroad.

Of the late varieties, "Brewer's Gold" and "Bullion Hop" had P.V.s of 109 and 95; and "Pride of Kent" came third with 92; "Sunshine Hop" had a P.V. of 75, and cropped at the rate of 19¾ *cwt.*; this variety shows a decided resistance to Downy Mildew and on this account is being much sought after by growers in the U.S.A. "Concord Hop" is another variety which shows definite resistance to Downy Mildew; it cropped at the rate of 19½ *cwt.*, but, possibly owing to the severe drought, gave a P.V. of only 54.* In the leaflet issued recently from Wye College† a table is given of its P.V. over 12 years at East Malling, which ranges from 89 to 64, the average being 73.

Mr. L. Fletcher, from the laboratories of Messrs. Wm. Younger & Co., Ltd., has kindly communicated the analyses, made from the pocket, of five of the new varieties grown by Mr. J. Nott, at Kyrewood, Tenbury Wells, Worcs. The analysis of "Early Choice," made on 27th January, 1948, was 6.00 *per cent.* α -acid, as compared with 4.55 *per cent.* at East Malling; the analysis, made on 27th January, 1948, of "Wye Field Golding" gave

* Mr. L. Fletcher, from the laboratories of Messrs. Wm. Younger & Co., Ltd., kindly supplied the analysis (from the pocket) of "Concord Hop" grown at East Malling in 1947; this gave the slightly higher P.V. of 60.

† E. S. Salmon: "Two New Hops, 'Early Choice' and 'Concord Hop.'" Leaflet, Wye College, Kent. January, 1948. Price 2s. 6d.

TABLE II (Abridged)*

Ref. No. or name of Variety.	Date of analysis.	α -acid per cent.	β -fraction per cent.	P.V. 10 α .
EARLY VARIETIES				
OT22	24/10/47	6.00	8.57	60
OJ47	4/11/47	5.58	9.47	56
"Early Choice" (FF21) ..	24/10/47	4.55	7.37	46
"Early Promise" (X35) ..	30/10/47	4.16	8.28	42
MIDSEASON VARIETIES				
"Brewer's Standby" (HH44) ..	5/11/47	10.46	8.98	105
"John Ford Hop" (WFA90) ..	4/11/47	9.82	10.19	98
WFA12 (clone-plant of "Northern Brewer" (WFB135)) ..	28/10/47	8.59	10.30	86
"Wye Field Golding" (WFC81) ..	5/11/47	8.38	9.67	84
X92a	4/11/47	8.09	10.56	81
"Malling Mid-season" (BB28)	29/10/47	7.77	11.51	78
OW76	23/10/47	7.40	9.31	74
OD18	28/10/47	5.67	12.25	57
HH50	5/11/47	5.36	8.39	54
"College Cluster" N15bis) ..	4/11/47	5.36	8.27	54
LATE VARIETIES				
"Brewer's Gold" (C9a)	23/10/47	10.91	11.29	109
"Bullion Hop" (Q43)	29/10/47	9.49	10.92	95
"Pride of Kent" (170a)	5/11/47	9.20	13.80	92
R3/100	23/10/47	8.90	11.68	89
AOM47	29/10/47	8.51	10.47	85
OR55	28/10/47	8.15	8.81	82
"Quality Hop" (OO63)	4/11/47	7.83	9.97	78
OW3	24/10/47	7.66	10.14	77
OB9	23/10/47	7.59	12.71	76
"Sunshine Hop" (V94)	24/10/47	7.52	8.85	75
OK40	29/10/47	6.85	8.10	69
"Fillpocket" (Z62)	29/10/47	6.79	9.56	68
ON78	24/10/47	6.76	7.46	68
"Brewer's Favourite" (OP21) ..	29/10/47	6.29	9.33	63
287a	5/11/47	6.28	8.15	63
Z74	4/11/47	5.83	8.85	58
AAA3	23/10/47	5.69	9.88	57
"Concord Hop" (OH44) ..	5/11/47	5.37	8.59	54

9.4 per cent., and the analyses, made on 18th February, 1948, of "Malling Midseason" gave 9.27 per cent.; of "Bullion Hop," 10.61 per cent., and of "John Ford," 11.31 per cent.; it will be seen, on referring to Table II, that these analyses are higher than those recorded for these varieties grown at East Malling; similar occurrences have been noted in past Reports.

It may be recorded here that Mr. Fletcher has reported that an analysis, made on 15th March, 1948, of a pocket of "Brewer's Gold" grown at Wye College in 1947, gave 10.37 per cent. α -acid, a P.V. of 104, indicating that this particular growth was keeping well in storage. A similar occurrence has been recorded by us (this *Journ.*, 1947, 100).

5. THE CROP OF NEW VARIETIES MARKETING BY REGISTERED GROWERS.

We have received information that in 1947 2,917 pockets, or approximately 4,375 cwt., of the new varieties were distributed by the Hops Marketing Board on behalf of 47 registered producers.† The figures for the past four years have been:—

1944	1,003 pockets	1,504 cwt.
1945	1,538 "	2,300 "
1946	1,868 "	2,800 "
1947	2,917 "	4,375 "

While the increase in 1947 was considerable, further planting of the new varieties is necessary to meet the brewers' requirements. As mentioned in the last Report, it appears probable that this further acreage will be found by the replacement of a portion of the Fuggle acreage with certain of the new varieties. Since 1944 it has been pointed out that there is need for the establishment of a Hop Experiment Station in the Weald of Kent, where approved new varieties can be tested in typical "Fuggle" soil. Under the new organization for hop research, which is sponsored by the Ministry of Agriculture, with the support of the Institute of Brewing and the Hops Marketing Board, provision has been made for this station and a site selected.

* The full table will be found in the Report as issued from the Research Station, East Malling, Kent.

† As regards Brewer growers, the Hops Marketing Board inform us that they have no information of the hops produced by them.

6. NEW VARIETIES RESISTANT TO *VERTICILLIUM* WILT.

Dr. W. G. Keyworth, of the Research Station, East Malling, Kent, has been carrying out research work over many years to ascertain whether any of the new (Wye) varieties show resistance to *Verticillium* wilt. The results obtained up to the present are given in three recently-published articles,* which should be studied by all growers whose gardens have suffered from this disease.

The variety found to be most resistant was "Nonsuch Hop" (OB53), which is of the highest brewing value, but gives only a low yield of some 13 to 15 *cwt.* *per* acre. The varieties OR55 and OJ47 which show a moderate resistance and give a high yield, are considered to possess the most commercial promise.

Preliminary brewing tests with OR55 and OJ47 have been carried out by Messrs. Whitbread & Co., Ltd., with satisfactory results.† Further brewing trials with both OR55 and OJ47 are being made at a number of breweries, and the results will be published in due course.

It has been decided by the Hops Marketing Board that propagation of OR55 and OJ47 on a large scale shall be undertaken, so that rooted sets may become available for growers who wish to plant up gardens where, owing to soil infestation by the *Verticillium* fungus, the cultivation of the Fuggle and other commercial varieties has become impossible. A committee appointed by the Hops Marketing Board has arranged for the work to be carried out at a commercial nursery in Kent, and it is hoped that distribution of sets may be possible in the autumn of 1948; applications for sets should be made to Mr. H. Christie, Medway House, High Street, Maidstone.

7. MACHINE PICKING OF HOPS.

In the last *Report* it was pointed out that experience in this country has shown that certain of the new varieties can be picked by machine with a higher efficiency than the

ordinary commercial varieties, and notes were given on the botanical, or structural, characteristics of the new varieties which render them suitable for machine picking. In an interesting article by D. Wigan (*Brew. Tr. Rev.*, 1948, 170), on the machine picking of hops in the U.S.A. and Canada, the following paragraph occurs: "The machine will definitely do a better job on certain kinds of hops. In England the new varieties, *e.g.* "Bullion" and "Brewer's Gold," seem to be ideal for machine picking because they are tougher and do not shatter so easily. I saw a machine in Chilliwack, B.C., doing an excellent job on these varieties, despite very heavy bines, and it might be of interest to mention that the yield was well over a ton to the acre."

In a recent article by Mr. F. J. Haas* it is stated that "Brewer's Gold" and "Bullion Hop" have been found to be "very productive in the Oregon area," and that "excellent results have been obtained with stationary machine picking." It is reported also of "Brewer's Gold" and "Bullion Hop" that "substantial commercial quantities are being grown in British Columbia and Oregon" and that "these are among the richest varieties grown commercially in the world."

8. BREWING TRIALS WITH NEW VARIETIES OF HOPS.

A Report on Brewing Trials with four new varieties (of 1946 growths), carried out by Messrs. L. Fletcher and R. P. McLintock, has been published (this *Journ.*, 1947, 249). The four varieties used were WFG19, WFB117, WFI36 and WFC69. WFG19 is a seedling raised in 1934 by "crossing" the "Canterbury Whitebine" with a male seedling (OB21) raised from "Brewer's Gold" (C9a), and is thus of the same parentage as "Northern Brewer" (WFB135) and "John Ford Hop" (WFA90). Analyzed on 17th March, 1947, WFG19 gave 6.92 *per cent.* α -acid and 9.35 *per cent.* β -resins; used in the inverse ratio of α -acid, only 16.7 oz. of hops *per* barrel were required for stability compared with 26.7 oz. of the control hop (Fuggle). The notes on physical examination were: "Appearance very good. Nice cones. Very good lupulin and pleasant aroma." In copper hop test: "Good flavour,

* F. J. Haas: "Report on New Varieties of Hops grown in the U.S. and Canada" (*Canadian Beverage Review*, May-June, 1948).

* (1) W. G. Keyworth: "Verticillium Wilt of Hops" (*The Brewing Trade Review*, 1947, 100).

(2) *Idem*: "Verticillium Wilt of the Hop (*Humulus lupulus*), II, The Selection of Wilt Resistant Varieties" (*J. Pomol.*, 1947, 23, 90).

(3) *Idem*: "Notes on Varieties of Hops resistant to Verticillium Wilt" (*Rep. E. Malling Res. Sta. for 1946*, 1947, 157).

† This *Journ.*, 1945, 39.

strong bitter, good nose, blends well." In dry hop test, 2 oz. *per* barrel (ground hops): "Excellent flavour, pleasant bitter, and not too strong."

It is believed that WFG19 will prove to be a heavier cropper than "Northern Brewer" or "John Ford Hop"; small plantings have been made by a few growers in Kent and Worcestershire.

Favourable reports were made also on WFI36 (of the same parentage as WFG19) and on WFB117 (a Seedling of II149 raised from "Brewer's Gold" (C9a)), when used in

the 1945 growths, were sent by 32 brewers, and on the 1946 growths by 41 brewers. A Table of Classification of Results by Variety, with Notes, was prepared, and this, which is reproduced below (Table III), has been published (*Brew. Tr. Rev.*, 1948, 335).

It is worthy of note that both "Brewer's Gold" (C9a) and "Early Promise" (X35) were found in many instances to be suitable for dry hopping. The brewers' reports contained information valuable alike to the brewer and grower, and it is hoped that these will be published.

TABLE III

BREWING TRIALS WITH NEW VARIETIES OF THE 1945 AND 1946 CROPS. CLASSIFICATION OF RESULTS BY VARIETY

Variety.	For Dry Hopping.			For Copper.		
	Favourable.	Doubtful.	Unfavourable.	Favourable.	Doubtful.	Unfavourable.
"Brewer's Gold" (C9a)	12	2	4	39	6	4
"Bullion Hop" (Q43)	4	1	9	27	4	8
"Early Promise" (X35)	7	1	1	19	7	0
"Brewer's Favourite" (OP21)	1	1	1	8	0	0
"Fillpocket" (Z62)	0	2	3	7	2	2
"Brewer's Stand-by" (HH44)	1	0	0	4	2	1
"Northern Brewer" (WFB135)	1	0	0	3	0	0
"Concord Hop" (OH44)	0	0	1	3	0	1
"Malling Midseason" (BB28)	0	0	0	2	0	0
"Pride of Kent" (170a)	1	0	0	1	0	0
"Quality Hop" (OO63)	0	0	0	1	0	0
"John Ford" (WFA90)	0	0	1	0	1	0
"College Cluster" (N15bis)	0	0	0	1	0	0
"Nonsuch Hop" (OB53)	1	0	0	1	0	0

blend or alone in the copper or in the dry hop test. The authors state that the average P.V. of commercial varieties in 1946 was 43; the P.V.s of the four new varieties tested were 69, 66, 65 and 59.

9. BREWING TRIALS WITH NEW VARIETIES OF THE 1945 AND 1946 CROPS.

The 1945 and 1946 growths of certain new varieties produced by commercial growers and marketed through the Hops Marketing Board were distributed by the Brewers' Society to brewers who had made applications for them for the purpose of carrying out brewing trials. The reports received from the brewers were transmitted by the Brewers' Society to the Institute of Brewing, who requested Dr. A. H. Burgess and the writer to summarize the information given.

Reports, of varying lengths, relating to

NOTES.

1. Improved stability of beer was noted in several cases and also in some of these cases a saving in the amount of hops used was recorded. The varieties concerned and the number of instances were as follows:—

"Brewer's Gold"	9 out of 54 reports.
"Bullion Hop"	4 " " 45 "
"Early Promise"	2 " " 27 "
"Fillpocket"	2 " " 14 "
"Malling Midseason"	2 " " 2 "
"Concord Hop"	2 " " 4 "
"Brewer's Stand-by"	1 " " 6 "
"Brewer's Favourite"	1 " " 10 "
"Quality Hop"	1 " " 3 "
"Northern Brewer"	1 " " 5 "
"Pride of Kent"	1 " " 5 "

2. The varieties "Fillpocket" and "Quality Hop" were found to be suitable for pale lagers; "Pride of Kent" was found to be suitable for one type of lager, but unsuitable for another type.

3. In a few cases the new varieties were favourably compared with continental hops (Auscha and Czechoslovakian).

4. In several cases criticism was made of the unsatisfactory state (weather damage, unsatisfactory packing, etc.) of the hops received.

10. THE CHALLENGE CUPS FOR NEW (WYE) VARIETIES OF HOPS.

In addition to the Challenge Cup offered in 1944 by Messrs. Barclay, Perkins, Messrs.

TABLE IV

ENTRIES FOR BREWERS' CHALLENGE CUP, 1947

Variety and grower.	Parish.	α -acid per cent.	β -resins per cent.
"Brewer's Gold" (C9a)			
George Beer & Rigden, Ltd.	Wye, Kent	9.58	11.42
J. Nott ..	Tenbury, Worcs.	8.36	14.89
G. A. & S. G. Calcutt ..	Goudhurst, Kent	8.30	13.59
F. Ivo Neame	Chilham, Kent	8.16	13.54
G. Nott & Co., Ltd. ..	Knighton-on-Teme, Worcs.	7.90	12.10
S. C. & J. H. Berry ..	Selling, Kent ..	7.88	11.77
S. C. Berry ..	Boughton-under-Blean, Kent ..	7.82	12.43
"Bullion" (Q43)			
*J. A. Worley ..	Chart Sutton, Kent ..	7.53	13.85
"Brewer's Gold" (C9a)			
J. A. Worley ..	Chart Sutton, Kent ..	7.45	13.89
Arthur Amos ..	Wye, Kent ..	7.38	14.02
"Bullion" (Q43)			
G. A. & S. G. Calcutt ..	Goudhurst, Kent	7.27	14.17
W. Rogers & Son ..	Horton Kirby, Kent ..	7.21	11.19
"Brewer's Gold" (C9a)			
R. C. Boucher ..	Teynham, Kent	7.19	12.27
F. Redsell ..	Boughton-under-Blean, Kent ..	7.02	11.11
"Bullion" (Q43)			
R. C. Boucher ..	Teynham, Kent ..	7.01	13.45
"Brewer's Gold" (C9a)			
R. S. G. Scott	Peasmarsh, Sussex ..	6.98	12.53
Blackmoor Estates ..	Liss, Hampshire	6.66	14.64

* Awarded Cup.

Guinness, and Messrs. Whitbread for the best annual growth of a new (Wye) variety of hop, a second cup, the Growers' Cup, was offered in 1946 by Members of the Executive Committee (1946) of the Association of Growers of the New Varieties of Hops. The competition is now divided into two classes: (1) the Brewers' Cup, for hops intended as substitutes for American hops; (2) the Growers' Cup, for hops intended to augment English commercial varieties. The fourth competition was held in the showrooms of Messrs. Wigan, Richardson & Co., 15, Southwark Street, London, on 27th and 28th January, 1948, the judges being Messrs. S. Combe, D. Le May and S. Myer.

For the Brewers' Cup 17 samples, from 14 growers, were entered, and for the Growers' Cup 10 samples from 7 growers. A list of the entries is given in Tables IV and V, together with the analyses, which were carried out at Wye College between 5th and 10th January, 1948, by Messrs. A. R. Tatchell and F. C. Thompson, under the direction of Dr. A. H. Burgess. The Brewers' Cup was awarded to Mr. J. A. Worley, for a sample of "Bullion Hop" (Q43). The Growers' Cup also was awarded to Mr. J. A. Worley, for a sample of "Brewer's Gold" (C9a).

As shown in Tables IV and V the winning samples of "Bullion Hop" and "Brewer's Gold" gave, respectively, a P.V. (10 α) of 75 and 95. The two samples of "Northern Brewer" had a P.V. of 98 and 88, and "John Ford," 95. All the growths exhibited gave a P.V. considerably higher than that of ordinary commercial varieties.

An exhibition of the samples entered for the competition was held also, on 5th and 6th February, 1948, at the College of Technology, Manchester, and was visited by a large number of Lancashire and Yorkshire brewers.

11. SUMMARY.

1. Of the 84 new varieties tested, 2 cropped at the rate of over 27 *cwt.* to the acre, and 13 at from 20 *cwt.* to 24 $\frac{3}{4}$ *cwt.*

2. The yield of 27 $\frac{1}{4}$ *cwt.* per acre given by OJ47 is believed to be unprecedented for an early variety.

3. The number of bushels (Imperial) of green hops required to the *cwt.* of dried hops varied from 69 to 131.

TABLE V
ENTRIES FOR GROWERS' CHALLENGE CUP, 1947

Variety and Grower.	Parish.	α -acid per cent.	β -resins per cent.
"Northern Brewer" (WFB135) J. Nott ..	Tenbury, Worcs.	9.79	12.93
"John Ford" (WFA90) Arthur Amos ..	Wye, Kent ..	9.53	10.74
"Brewer's Gold" (C9a) *J. A. Worley ..	Chart Sutton, ..	9.50	10.83
R. G. Probert	Peasmarsh, Sussex ..	9.26	11.76
E. E. Taylor ..	Kingsdown, Kent	9.01	12.43
"Malling Mid-season" (BB28) J. Nott ..	Tenbury, Worcs.	8.93	11.69
"Northern Brewer" (WFB135) Arthur Amos ..	Wye, Kent ..	8.82	9.79
"Bullion" (Q43) J. A. Worley ..	Chart Sutton, Kent ..	8.30	14.46
"Brewer's Favourite" (OP21) G. A. & S. G. Calcutt ..	Goudhurst, Kent	7.53	8.50
"Early Promise" (X35) R. C. Boucher ..	Teynham, Kent	6.19	8.11

4. High analyses were obtained with many of the new varieties, "Brewer's Stand-by" giving a P.V. of 105, and "Brewer's Gold," a P.V. of 109.

5. It is pointed out that the crop marketed of the new varieties (4,375 *cwt.*) falls considerably short of the brewers' requirements. It is considered probable that the required acreage will be found by the replacement of a portion of the Fuggle acreage with certain of the approved new varieties, and that the proposed hop experiment station in the Weald of Kent will further this.

6. Notes are given on (1) certain varieties resistant to *Verticillium* wilt, and (2) the machine-picking of hops.

7. The results of brewing trials with four new varieties are given. Brewing trials with new varieties of the 1945 and 1946 crops have been published; in many instances both "Brewer's Gold" and "Early Promise" have been found to be suitable for dry hopping; three varieties were found suitable for lagers.

8. Particulars are given of the competitions for the two Challenge Cups offered for the best 1947 growths of a new (Wye) variety of Hop. Among the excellent commercial growths exhibited, a sample of "Brewer's Gold" gave a P.V. of 96, "Northern Brewer," a P.V. of 98, and "John Ford Hop," a P.V. of 95.

MEETING OF THE LONDON SECTION HELD AT THE HORSE SHOE HOTEL, TOTTENHAM COURT ROAD, LONDON, ON MONDAY, 8TH MARCH, 1948

Mr. B. M. BROWN in the Chair

The following paper was read and discussed:

FROM CASK TO CONSUMER

By J. W. SCOTT

IN this paper it is proposed to examine storage conditions and methods of service of draught beer as they exist today, and to offer suggestions for the improving and speeding up of such service, the importance of which cannot be exaggerated if draught beer is to maintain the popularity it deserves.

Before proceeding to the main theme, it may be strongly urged that the average brewer, and particularly the under brewer,

has a very restricted knowledge of the problems and difficulties with which a licensee has to contend. This is probably due to two reasons: firstly, the brewing staff is usually too fully occupied to spare a member to study such problems; and secondly, many brewery companies have few, if any, managed houses, therefore even if the brewer had the time he might have difficulty in enforcing his ideas. In the course of a brewer's training, a period

should be set aside to be spent on licensed premises where experience of actual working conditions and the problems with which a licensee has to contend could be gained at first hand. Some firms employ brewers attached to the brewing staff to supervise their retail cellars; they thus have a great advantage over those who leave the reputation of their products entirely at the mercy of the licensees themselves. Others rely on the outside staff, which has rarely had specialized training in the problems of cellar management and equipment or in methods of dispensing.

If brewery companies employ beer inspectors they should be under the control of the brewing staff, to whom they should make daily reports, and so form a valuable link between the producer and the retailer and enable the brewer to obtain first hand knowledge of the way his products are treated and how they respond, and should there be complaints they are more likely to be quickly and efficiently dealt with.

The training of licensees should also be more thorough, and cover the process of brewing in some detail, so that sound theoretical grounding could be obtained as well as practical training. The Licensing Commission, in its report, stresses the importance of a much wider training of publicans, and a start has already been made in this direction by the London brewers. Nevertheless, however good this general training may prove, there will always be room for further instruction with the individual firm's products and methods.

Many improvements in plant and processes connected with brewing have been made during the last 50 years; but what improvements have been made in the serving of draught beer since the advent of the vent peg—a most temperamental object, especially these days—or the beer pull, which requires a flat beer if the glass is to be filled reasonably quickly without too much waste? Numerous attempts, chiefly by engineers, have been made to obviate some of the publican's difficulties, but few have met with success, due to the peculiarities of beer and insufficient opportunity and encouragement or data to experiment. The foremost difficulty is to control the condition of his beer to enable the publican to serve brilliant beer in the best possible condition at maximum speed, whether trade is brisk or quiet.

Before offering suggestions on how to try to attain this most difficult goal, cellar conditions may first be examined to see if anything can be done to make them more suitable for beer storage. Most cellars have brick floors, and almost invariably the pointing has been eaten away. To repoint would only be a temporary repair, but a 2-in. layer of best quality "Granolithic" would make a first class job, and the opportunity should be taken to ensure adequate falls to the drain or pump-emptied sump. An adequate water supply is of primary importance, as not only is water needed for cleaning purposes but it can be used very effectively to keep down the temperature of the cellar by ensuring that the floor, casks, and walls if possible, are always damp. Dampness alone, however, is not enough, as the cooling effect is obtained by evaporation, which can only be brought about by thorough ventilation of the whole cellar.

It is difficult to obtain sufficient air changes *per* hour unless the natural ventilation is assisted by a well placed fan, which may have to be coupled to ducting to obtain the even distribution which is so necessary. Ducting should, however, be avoided for obvious reasons, even if it means installing additional fans. All ventilators should be conveniently adjustable, and if it is possible to connect one or more to a chimney breast a very effective induced draught can be obtained. Much can often be done by partitioning off large cellars to isolate heating chambers, sections that are not used, passages *etc.*, in order to make temperature control easier.

Apart from the difficulty of obtaining cooling plants, the type of condensing unit known today for use with alternating current is not advocated because it is hoped that suitably sized hermetically sealed condensing units will shortly be available. At the present time these units are only of very small capacity, but larger ones are being considered. The great advantage of this type of unit is that maintenance has been reduced to the absolute minimum, since the condensing unit has the compressor and electric motor hermetically sealed in a welded steel case, and all moving parts are permanently lubricated. The need for crank-shaft seals and driving belts is eliminated and the possibilities of refrigerant leaks or of the ingress of air or moisture which cause

so much trouble in existing plants are very remote.

Refrigeration is considered by many to be an expensive luxury, and probably in many cases this is true. It must not be forgotten, however, that quick and waste-free service of well conditioned beer cannot be achieved if the temperature of the beer rises whilst in the cask or whilst being dispensed. Successful dispensing is only possible when the minimum of gas comes out of solution, and refrigeration can often be very helpful in attaining this end by preventing a rise in temperature with accompanying loss of gas and fobbing.

One of the most difficult problems since the practice of drawing beer direct from the cask in the serving was superseded, has been to find a material suitable for piping, a material possessing flexibility and having no deleterious effect on beer; all that has been possible till recently is to compromise. Now, however, the considered opinion may be expressed that the material has been found; it only remains for the Government to release it in sufficient quantities. This material is "Polyethylene," or "Polythene" for short. It is a straight plastic, containing none of the plasticisers which are the source of so much trouble in the more commonly known polyvinyl chloride piping. It is inert to beer, is non-absorbent (the fact that it is used almost entirely for the insulation of submarine cables should be a good enough recommendation on this point), it is sufficiently flexible, will not kink, providing the wall thickness is adequate, can be moulded, is easily cleaned, will stand hot water, is light in weight, resistant to rough handling (even to an occasional cask being rolled over it), its life should be very considerable, and it is not expensive.

Owing to polythene being in such short supply, other plastic materials are being offered, some of them having a very attractive appearance when new. One of these, polyvinyl chloride, in its pure form is non-flexible; plasticisers are added in varying quantities according to the flexibility required, and these often cause undesirable flavours and discolouration of the piping, due to absorption by the plasticisers, a defect which is often covered up by adding pigments. Should such pipes be out of use, they go sour. There is probably at the moment nothing in the plastic range other than polythene which

is inert to beer, and is at the same time flexible, and there is no doubt that when supplies become plentiful there will be other applications in the brewing industry.

For obvious reasons, irrespective of how good pipe installations in cellars can be, the less piping there is used the better, and in many cases (particularly if the serveries are over the cellar) ceiling pipes, which seldom serve a useful purpose should be done away with, either by moving the thravls nearer the dispensers above or by supplying extension pipes to be added when necessary to the drop pipes. The use of extension pipes is to be preferred to the use of long drop pipes, as pipes which are not in use get dirty more quickly than those that are continuously in use, unless they are cleaned immediately after disconnecting. Where practicable, moveable thravls should be used, enabling the maximum amount of beer to be assembled at any point; if not required, they can be stored out of the way. This method ensures the shortest possible drop pipes.

One of the golden rules when installing beer-raising equipment is to ensure that there is no variation in the bore of taps, pipe work, and other fittings. If it is decided to use $\frac{1}{2}$ -in. bore piping, then all taps and fittings should be $\frac{1}{2}$ -in. bore, since if beer has any condition in it at all, some gas is bound to come out of solution if the flow is increased from a small to a larger bore. A typical example of this is the cask tap, which in its most common form is probably the worst tap ever designed for a specific purpose. Beer flows through the small holes in the hop trap into the large body of the tap and then through a narrow slot in the plug, from where it turns a right angle into the much larger bore of the tap outlet. Another example which has become more apparent since plastic pipes were introduced, is the metal tail at each end of the pipe, which is often $\frac{1}{2}$ -in. bore, whereas the pipe is $\frac{5}{8}$ -in. Apart from the fact that gas will come out of solution when the flow of beer passes from $\frac{1}{2}$ -in. into $\frac{5}{8}$ -in. bore, it can hardly be considered economical to pay for $\frac{5}{8}$ -in. piping when its capacity is limited by the tails to $\frac{1}{2}$ -in. bore.

Some may consider that this exaggerates the importance of bore variation, but the frothing and waste formed when clearing pipes after connecting up a full cask is due almost entirely to gas having come out of

solution as the beer flows from the cask to the dispenser. Frothing may persist for a considerable time, and even gas locks may form if the beer happens to be in good condition. A length of transparent piping in the ceiling run or drop pipe will provide further proof should this be necessary.

Last, but not least important is a really comprehensive cellar management instruction card, which, if intelligently compiled can be of great assistance to the licensee and the brewery company. The one cannot claim lack of knowledge if such instructions hang in his cellar, and the other hopes that if all licensees carry out the instructions the beers will be more consistent.

There seems little likelihood, even when supplies of beer become normal again, that peak periods of trade will diminish, owing to the uneven distribution and in many cases unsuitable design of licensed premises, but until districts are adequately catered for the peak periods will remain and must be provided for. The method of dispensing beer most commonly used today was designed, not for the rush periods, but for the more uniformly busy house open most of the day, and with unlimited supplies. This refers, of course, to the use of pulls, or pressure systems where the beer is dispensed under pressure from a tap into the glass. The fact that these systems are incapable of fast service without making considerable waste is only too obvious, but much can be done to remedy this state of affairs, and the author proposes now to offer suggestions for the speeding up of service without the necessity for flooded trays and drip bowls, wet counters, glasses, floors, *etc.*, not to mention the fatigue caused when using pulls.

In order to accelerate the dispensing of beer, it must be admitted that with most types of dispensers it is necessary to flatten the beer in the cask in order to make this possible. Manufacturers, aware of this, have provided their dispensers with small bore outlets, or so-called "sparklers" in order to make the glass of beer look more attractive by adding a very temporary false head; but it is this small bore outlet which is largely responsible for the slowness of service and the overflowing of glasses, because the beer is discharged at such high velocity, usually into the bottom of the glass, that any gas remaining in solution is knocked out of the beer in the form of foam.

Beer pulls were designed to deliver a half pint at one pull, but owing to the large arc of travel of the handle which only suits those who are just the right build, coupled with the effort that pulling requires (increased owing to the resistance of the restricted outlet), most operators make two or more short strokes to fill a glass. Since the angle of pull is much more convenient at the beginning of the stroke, the speed of stroke is then greater, so that the beer is ejected into the glass at a much greater velocity, with considerable agitation of the beer. The manipulating of beer pulls in this manner makes the flattening of the beer in the cask even more essential.

It may be suggested that the following alterations would make the beer pull more adaptable to quicker service:

1. Improve the angle of pulling. One way of doing this is to incline the handle slightly towards the customer.
2. The rising main from the cylinder and the outlet tap should not have a bore of less than 9/16 in.
3. Adjust the stroke of the piston so that slightly more than one half-pint could be discharged.
4. Fit an extended sleeve to the piston, or make other arrangements to close the main outlet from the cylinder when the level in a half-pint glass is $\frac{1}{4}$ in. from the rim. A further movement of the pump handle will eject a jet of beer into the glass which will agitate the surface of the beer and top up at the same time.

With the capacity of the cylinder adjusted to give slightly more than one half-pint, the operator would have no difficulty in filling the glass at one stroke without overflow, as it is much easier towards the end of a stroke to control a jet of beer rather than a full bore flow. With the main outlet of large bore, there would be less resistance to pulling, and the beer would flow into the glass quietly. An automatic system for raising beer to the dispenser is very much to be favoured, choosing either an air pressure system or an electrically operated pump. Which of the two is more satisfactory may be left to personal judgment, but brief reference may be made to both methods.

The prejudice against compressed air is

tremendous, the chief criticisms being soft palate, too gassy beer, complicated installation, necessity for extra strong casks and risk of leaks, the need for reducing valves and filtering of the air, and inability to serve if the electric supply fails. To such criticisms it may be replied that there is no kinder way of raising beer, and if the beer tastes soft it is due to a badly designed dispenser or outlet tap, causing excessive agitation in the glass. Air cannot cause softness unless it is intimately mixed with beer, or is in contact with it for a considerable time. The beer may become too gassy, but not if excessive condition can be automatically released at the cask.

The air compressor should be of the low pressure type, delivering air at, say, 8 to 10 *lbs. per sq. in.*, which is all that should be necessary to lift beer to a ground floor servery. At such pressure, casks should not be unduly strained and reducing valves are not required. The compressor should be capable of hand turning in case the electric supply fails; as an alternative to an electric motor, compressed air can be produced by the use of water pressure—a popular method in Scotland. There are so many satisfactory methods of filtering air that they need not be dealt with here. If the components comprising a compressed air system are carefully and intelligently chosen, this method of beer raising will be found to be simple and trouble-free, with the great advantage of maintaining condition in the beer longer than any other method.

The raising of beer by centrifugal pump never appears to have received much attention, probably because it is thought that the agitation by the impeller would cause frothing and possible gas locks, etc. The author has not found this to be the case, but on the contrary finds it very satisfactory, providing that the output is always sufficient to ensure that the rising main is completely filled with beer, however fast the service.

The diaphragm pump may have a greater appeal, because in one form the diaphragm ceases to pulsate as soon as the outlet is closed, so that agitation takes place when beer is not being drawn off. The criticism of these pumps is that they do not give a smooth flow unless two or more diaphragms are used, but some smoothing out can be effected by the use of an air bottle. Initial cost is greater than that of any other method

of beer raising, but priming is unnecessary, which means that the pumps can be placed in any convenient position, in bar or cellar. The centrifugal pump, on the other hand, should be at thrawl or floor level; the latter position is perhaps to be preferred as the suction pipe is very short, and the possibility of sucking air and the disastrous consequences that follow are minimized.

Failure of the electric supply in the case of either pump would be a serious matter; a stand-by air or CO₂ bottle with reducing valve and a few air connection fittings to screw into the cask vent hole is as satisfactory a device as any for meeting such an emergency. A small petrol generator set is another possibility, or low voltage motors could be used, in which case they would be supplied from accumulators and trickle charger. If it is necessary to raise beer above ground floor level, *e.g.* to a club room or dining room, the electric pump comes into its own as it is far superior to any other method.

No matter what system of beer raising is employed, whether it be pulls, pumps or pressure, it is a very great advantage to be able to ensure that the condition in a cask never exceeds the requirements of the system in use. If a vent peg is employed it is sheer luck if the correct condition is obtained, and the attention it requires is considerable. There is much to be said for a properly constructed automatic blow-off valve which will reduce the condition to a pre-determined pressure. If pressure raising is not in use a vacuum valve should also be incorporated. This is invaluable when pulls or pumps are used, as the cask remains sealed so long as beer is not being drawn; no gas is, therefore, lost, as is the case when the vent peg is eased or withdrawn, since the valve automatically closes every time the flow of beer from the cask ceases, thus maintaining the condition for a longer period. The vacuum valve for use on casks has been on the market for years, but in spite of its undoubted value, even without the blow-off valve, it is seldom used.

To design a dual valve that can be relied on to carry out these two functions is not easy, for it must be foolproof, cheap, quickly cleaned, unaffected by foam, hop seeds or leaves and capable of reducing the pressure from, *e.g.*, 15 *lbs. per sq. in.* (which may easily be exceeded after a long journey in

warm weather) to around 5 lbs. *per* sq. in. without causing foaming or waste. It can be done, and the advantages to be gained are considerable, not the least important being the elimination of the human element. In passing it may be suggested that the practice of coupling all casks together to a common gas receiver, so conserving the gas given off and incidentally reducing the number of condition valves to one, is worthy of careful consideration.

Neither of the beer-raising systems just referred to will give complete satisfaction if the beer dispenser is, in effect, a tap on the end of the rising main, as the sudden reduction of pressure of the beer as it leaves the tap, usually at high velocity, cannot fail to cause considerable waste if the beer is in reasonable condition. Since this waste flows down the glasses, often over the fingers of the operators, or on to a tray, there should be only one place for it, the drain.

Passing now to the requirements of a successful dispenser, it must reduce the pressure of the beer to atmospheric as slowly as possible, the discharge being of large volume and slow velocity. Only by this means is it possible to fill a glass quickly with beer in good condition without frothing and waste. It must also fulfil the following requirements: no means of utilizing waste should be provided; a measured quantity should be dispensed; it must be foolproof and capable of being operated without tuition or fatigue. Further, it must be easily and quickly cleaned, and need the minimum of maintenance; it must be easily installed, without cutting of counters or shelves. It should take up minimum space under the counter, leaving top of counter clear, and the discharge nozzle should be taken to the glass, not *vice versa*, thus avoiding handling and spilling. The beer must not go flat if it remains in the dispenser, but it should be possible to agitate the surface of the beer in a glass if there is insufficient head. A counter should indicate amount sold and contents of cask, *etc.* Lastly, cost should be competitive. The description of the apparatus designed by the author to dispense "controlled condition" beer, raised by either air pressure or pump, and covering as far as possible these requirements, is as follows:—

Beer under pressure from the supply is directed alternately to opposite ends of a

cylinder in which is a piston, moved by the beer. Movement of the piston produced by the incoming beer at one end of the cylinder forces out to the discharge nozzle beer which has previously entered at the opposite end of the cylinder. A plug type change-over valve directs the beer to alternate ends of the cylinder. At the top end of the valve is a projecting hollow stem to which is attached a flexible hose and outlet nozzle, through which dispensing takes place. Mounted on this stem is a toothed pinion which is engaged by a toothed quadrant mounted on a pivot carried by the valve casing. A small movement of the control handle attached to the quadrant turns the valve through a right angle from one position to the other. The cylinder is arranged with its axis at right angles to the axis of the valve, and is provided with end plates in each of which is an "L" shaped port communicating with the change-over valve. The piston has a face joint at both ends so that when it moves to either end of the cylinder it makes contact with and seals the "L" shaped port which communicates, *via* the valve, with the discharge hose.

It will be seen that, in effect this apparatus discharges a measured quantity, and that when the piston has displaced this amount it automatically seals the outlet, so that there is no need to return the control lever to a "neutral" or "off" position; the beer on the other side of the piston, which is now awaiting discharge, cannot go flat because, until the change-over valve is moved again, the beer remains under pressure from the cask. Even if it were possible to do so it would not be policy to dispense an accurate quantity of beer, as room must be left for the head, which will vary with the condition, so the apparatus is designed to give just under one half-pint. In order to top up the glass, and if necessary agitate the surface of the beer to increase the head, a flexible pipe is connected directly from the beer supply pipe to a thumb-operated valve situated just behind the main discharge nozzle. From this valve a jet of beer under pressure can be directed into the glass.

Gas is prevented from coming out of solution when the beer enters the cylinder from the supply pipe, since there is very little pressure drop owing to the resistance of

the piston dispensing the beer on the discharge side through the large bore flexible discharge pipe. Agitation is reduced to the minimum as the flow into the glass is of large volume but slow velocity, and can be easily directed down the side of the glass. The operator has no control over the flow from the discharge, it being full bore until the measure is dispensed. This is very important, particularly in the case of pressure or pump systems, as if an outlet is throttled down by means of a tap, agitation is greatly increased, because the beer is discharged at a much higher velocity but much reduced volume, usually against the side of the nozzle, owing to the restriction of the partially closed valve. This results in a very rapid pressure drop causing gas to come out of solution in the form of fob.

The reason for using a flexible hose discharge is to reduce spilling as much as possible by making it impossible for the operator to handle the glass or glasses whilst dispensing.

The dispenser is secured by two wing nuts to a plate attached to the underside of the counter, it being only necessary to disconnect the supply pipe and slack off the wing nuts to enable the complete apparatus to be removed. The control lever extends from beneath the counter, and just above it is a short flexible discharge hose and nozzle which is clipped to the edge of the counter when not in use. Glasses to be filled are placed on the counter within range of the discharge hose which can be held in either hand, the other hand operating the control lever which, as mentioned, is not returned to an "off" position when the glass is full, as the dispenser shuts off automatically. Topping up can take place whilst the main discharge is still flowing, once the operator discovers how long it is necessary to press the valve to obtain a correctly filled glass. To fill a pint glass the control lever is moved twice; any number of pints can be filled at the rate of approximately 12 pints *per* minute, without waste. If less than half a pint is required the topping up device is used by itself.

For the convenience of the licensee an automatic counter is fitted which is useful for indicating when to stoop, the quantity in cask, or the quantity sold.

One of the main objects in designing this dispenser has been to obviate waste, and there is no facility for its disposal should any

be made, as experience proves that if such facilities are provided they are invariably abused. There is probably no method of waste disposal which is satisfactory and free from abuse, except where it is returned to the brewery, but such a scheme would entail complications in a large firm. To avoid waste would seem to be the only solution to this problem. Providing that the beer pipes are of correct bore and are as short and clean as possible, and the dispenser is efficient, the biggest remaining source of waste occurs when the cask is first drawn on or when it blows. In order to obviate this a paper-type filter is used, fitted with a by-pass valve, easily accessible beneath the dispenser. The first gallon or two from a new cask and the first few pints each session are filtered as also is the last gallon or so before blowing. Delivery from the filter is intentionally adjusted to slow down service so that there is no encouragement for the operator to continue filtering any longer than is necessary.

The final suggestion for aiding quick service is that dispensers should be spaced along the serveries in single units, not in groups of from two to six, as is so often seen. This spacing obviates congestion and delay in serveries and distributes the customers more evenly, and also spaces out the casks in the cellar.

In order to prevent delay in changing over to a full cask when one has emptied, it is often the practice, particularly in the busiest houses, to instal spare dispensers. This always seems an expensive method, for it entails extra expenditure on dispensers, pipe work and cleaning, and probably takes up valuable counter and shelf room in the serveries. A far simpler device is to make use of solenoid valves controlled from the servery. They are reliable, but if they go wrong it only takes a matter of minutes to disconnect them and couple direct to the cask.

Many people think that the only beer worth drinking is naturally conditioned draught beer, but there is no doubt that, prior to the war, chilled and filtered beer—both draught and bottled—was rapidly gaining in popularity because it offered a more consistent drink and was less trouble to the brewer when out in the trade. This will no doubt be the case again when conditions return to normal, unless the industry

as a whole studies the problems of licensees and offers them a more thorough training, so that they have a wider knowledge of the article it is their job to sell. A well-trained licensee is, however, at a disadvantage if his equipment is out of date or unsuitable for his type of trade; it is up to the brewer, therefore, who knows only too well the peculiarities and complexities of beer, to make known his requirements for the improvement of the service of draught beer so that the mechanically minded have more definite data on which to work.

There is no justification during these difficult times for incurring capital expenditure, but the time will come when the beer drinker will not go in search of the house with beer to sell, but rather the one that serves the best beer, irrespective of whether it happens to possess luxurious furniture and decorations. There is an excellent opportunity now to experiment and to revise ideas on beer dispensing. The nature of draught beer makes it very vulnerable when subjected to improper treatment, but when intelligently handled it responds in a remarkable manner, and much can be done to ensure consistently good results by the provision of proper facilities and by attention to detail.

DISCUSSION

The CHAIRMAN (Mr. B. M. BROWN) emphasized the importance of the proper distribution of beer to the customer, and said that perhaps some people had felt for a long time that the technique of treatment of beer in the public house had not reached the level of its treatment in the brewery. Two very important factors were outstanding in connection with the service of beer. One was the reduction of waste, and the other was the paying of proper attention to the temperature at which the beer was served. He agreed that about 58° F. would be satisfactory, but doubted whether the temperature could be kept down to 58° F. during the summer months without some mechanical system of cooling. Had the author any experience of the use of a cabinet cooler in the bar, as opposed to refrigeration in the cellar?

Mr. C. L. DOWNING asked if the author had considered using the outside air for cooling cellars, because it was often of low temperature and could be used for such cooling at very little cost.

Mr. W. E. HORNSEY pointed out that the problem of training licensees was one to which brewery companies had given much attention during the last few years, and there were schools for that purpose in Blackpool and London. As a practical brewer, he was opposed to the use of polyvinyl chloride for piping, but vendors had approached the tenants first and managed to get it installed. He had yet to be convinced that a plastic pipe of any description would supersede

the stainless steel pipe with internally polished bore; the latter type did not sag and could be kept off the floor. Welcoming the author's remarks on the electric engine *versus* air raising or CO₂ raising, he said that in a very considerable experience of air raising he had seen many plants discarded. Again, after using a CO₂ system for 12 months, the tenant in a fairly large house had discarded it, as it was using one tube of gas *per* barrel of beer; that was a common experience. He held that an expanding ring engine which came into contact with beer was wrong, as a beer engine of that type collected amorphous matter; cases had been brought to his attention in which the expanding ring had seized up and the lever had been broken. He maintained that the original all-metal bucket engine was the most satisfactory. If it were contemplated that the machine described by the author should serve as a measure, there would probably be difficulty, because any measuring device must be sealed by the weights and measures authorities. Again, if the beer had to be filled to the tops of the glasses, the creamy head was bound to be lost.

Mr. M. S. HEYCOCK asked the author's opinion as to the temperature at which beer should be consumed. Should it be colder in hot weather and warmer in cold, or at the same temperature all the year round?

Mr. T. WRIGHT said that, in view of the variety of beers to be served, however long the counter might be, customers would go along to the appropriate pumps if they were spread along the counter, and the bar would become far more congested than when pulls were in set positions. One type of electrical pump would probably serve two or three varieties of beer, whereas the centrifugal might serve only one; with five different types of beer there would be needed at least three double system electric pumps, or probably five centrifugals. The expense entailed in either case would probably be far greater than when using the present-day beer pull, which would continue in operation for years, needing very little repair and little maintenance.

Mr. H. G. SPILLANE asked whether it was possible for the author's dispenser to be regulated to serve half-pints of mixed beers.

Mr. W. J. WATKINS said that the only matter on which he disagreed with the author was in regard to cellars, for he did not like damp cellars, nor the idea of having to use water to absorb heat. The requirements could be met otherwise by harnessing the elements outside at suitable hours of the night or day; the temperature could be, and was being controlled in properly constructed cellars, which remained absolutely dry. Moisture favoured the development of various organisms, no matter how good the cellar was, because infection was carried in on the exteriors of casks.

Mr. R. T. STOREY asked for the author's views on glass washing, and on the effect of an unwashed glass on beer.

Mr. J. GRANT thought that most people would agree that the flatness of beer in many houses was due to casks being on ullage far too long. Thus, a cask might conceivably be in use four days before it was empty, and on the fourth day the beer was not worth drinking. In order to overcome that trouble, would it be desirable,

instead of having one pull for each cask, to have at least two, where necessary, to ensure emptying in one day?

Mr. Scott replied that the outside air could be used for cooling in conjunction with water sprayed over casks, walls and floors, as had been mentioned early in the paper. Five or six changes of air *per* hour were necessary to obtain the evaporation necessary for cooling. The method was well worthy of consideration, particularly as cooling plants were so expensive; however, in many cellars it was not possible to spray water, because there was no sump or drain, and the walls were not suitable. He had suggested spraying merely to assist temperature control without going to the expense of structural alterations or of other means of making a cellar suitable for the use of stored-up conditioned air. He had not meant sprinkling with water to make the place merely damp, but wanted to see the hose turned on the walls and floor in order to get it really wet. Moulds would have no opportunity to develop, if the hose were used regularly. The practice of drawing in cool air from outside during the night to cool down the cellar was only worth while in properly constructed cellars, as in the ordinary cellar the cooling effect was only of very short duration.

He had not attempted to go too deeply into the question of refrigeration. If the dispensers were some distance away from the cellar, so that there was long piping, there would be a temperature rise, even though the beer were kept reasonably cool in the cellar. If an electric refrigerating plant could be afforded, he was in favour of the hermetically sealed type which would soon be available. If possible, the blower type evaporator should be used—preferably without ducting, although in some cellars ducting might be necessary to avoid the expensive use of several blowers. He did not believe in the convection type of cooling; distribution was too uncertain. He thought beer temperature should be approximately the same throughout the year, *viz.*, about 58° F.

The machine he had described did not give a definite measure, though it was attempted to approach it closely; he could then give a head, or could fill the glass right to the top by means of the topping-up or agitating device. It was almost impossible to design a machine to give a precise measure because of the varying condition in the beer, which covered a fairly wide range when a vent peg was used. If less than a half-pint of a particular beer was required, the topping-up

device was used, the beer being directed down the side of the glass to avoid excessive agitation.

There were no rings in the system he had described; there was a loose-fitting or floating piston. When beer was introduced at one end of the cylinder the piston was pushed to the other, displacing a large volume at slow velocity. The piston was made of Perspex. The pipes normally did not sag, as they were made of Polythene. He agreed that polyvinyl chloride pipes should be avoided. With regard to stainless steel, the telescopic stainless steel pipe was, he thought, dirty and very easily damaged; the alternative collection of universal joints formed traps for dirt, and both types were very expensive.

With regard to spacing, he had merely meant to imply that where there was one main popular variety he would then spread the dispensers. It would be a good idea to couple up two or more pulls to a cask in order to empty it reasonably quickly. He had not seen an electric pump with one diaphragm which would give a smooth flow, due to the pulsation of the single diaphragm; the resulting agitation was very troublesome with a well-conditioned beer. Two diaphragms working together off the same cask was a fairly good arrangement, but the high price probably ruled this out. The centrifugal type of pump which he had used cost about £7, not including the price of the dispenser; this varied according as to whether just an outlet tap was used or an apparatus such as he had designed. On the other hand, a single-beer pull would cost from £15 to £25.

Glass washing presented a big problem. There were many glass-washing machines on the market which cleaned individual glasses, but in his view they were useless, except, perhaps, when the house was slack and glasses could be put through singly. The glass-washing problem should be tackled on a big scale; the process must be automatic, the glasses being fed on to the machine and coming off automatically. There must be a certain amount of friction, especially round the rim; mere spraying was insufficient. The varied types of glasses made the problem really difficult. New licensed houses should have a room set aside for glass washing. It was satisfactory as a temporary measure to have two bowls butting against each other, so that water was run continuously into one bowl and overflowed into the next, and out through a small hole into the waste from the second. The operator would take several glasses at a time, plunging them into the second bowl first, and then rinsing them into the fresh water bowl.

ABSTRACTS

I.—MATERIALS AND ANALYSIS

Amylases of Barley and Malt. H. LUNDIN (*Brewers' Digest*, 1948, 23, No. 10, 55-57 and 64). The author reviews the recent results obtained by various investigators upon the amylases of barley and malt. These enzymes were isolated in conjunction with albumins by extraction of the materials with dilute salt solution followed by alternate precipitations with different concentrations of ammonium sulphate and dialysis. On continued purification both the α - and β -amylase activities of malt increased until maximum values were attained. The purified albumins from barley and malt showed identical sedimentation constants in the ultracentrifuge but considerable differences were found in their enzymic activities. Both barley and malt displayed β -amylase activity, although this was higher in the latter but α -amylase was present only in malt. Aqueous malt amylase solutions remained stable for several weeks at 1° C., but after a longer period a small precipitate was formed which showed a higher enzyme activity than the solution. Since the α -amylase is precipitated to a greater extent than the β -amylase it is possible that fractional precipitation at a low temperature would enable these enzymes to be separated. By ultrafiltration through a collodion membrane the residues obtained showed a higher enzyme activity than the original material and when these were freeze-dried stable powders completely soluble in water were obtained but up to about 24 per cent. of the activity was lost. Attempts to separate the enzymes by electrophoresis failed because the migration velocities were practically identical over a wide range of p_H values. A comparison of malt α -amylase with the crystalline α -amylase isolated from pancreas showed considerable differences between them in their dextrinising power and optimum p_H value. T. J. W.

Oxidizing Enzymes in Brewing Materials. IV. o-Phenylenediamine Oxidase in Rice, Wheat and Maize. J. S. WALLERSTEIN, M. G. HALE and R. T. ALBA (*Wallerstein Lab. Commun.*, 1948, 11, 221-227). The activity of this enzyme, previously examined in malt (this *Journ.*, 1948, 39), has now been investigated in a number of other cereals. The activity in wheat, which is maximal at p_H 3.0, was approximately twice that of barley, but samples of white and yellow maize and of rice showed no activity in the raw grain. Wheat showed a substantial increase in the oxidase activity as a result of malting, and similarly a

limited development was observed on malting maize and rice; in all cases acrospire and rootlets showed a high concentration of the enzyme. Kilning destroyed some 40-50 per cent. of the activity of the green malt; there was some degree of stability under mashing conditions, a fact in agreement with the behaviour of the corresponding barley enzyme. I. A. P.

Biological Inhibition in Malting. P. FROESCHEL (*Chim. et Industr.*, 1948, 60, 94). All seeds contain germination inhibitors. If these are removed the seeds germinate more rapidly, and if the extracted inhibitors are added to other seeds their germination will be retarded or even prevented. Barley steep liquor contains an inhibitor, and if this liquor is concentrated and sprinkled on the germinating barley, rootlet growth and respiration can be restricted to an extent sufficient to diminish malting loss by 2 per cent. Solutions of inhibitors from other types of seed can be used to produce the same effect. A. A. D. C.

Practical Trends [in Brewing]. M. GOCAR (*Chim. et Industr.*, 1948, 60, 94). The author first describes the method of preparing a cold water extract of hops for use in dry-hopping bottom fermentation beers, and refers to the lining of metal beer tanks with a "hopped pitch" (*poix au houblon*) in order to improve the flavour of the beer. A means of improving the fermentation of low-gravity beers is then discussed. Finally, the oligodynamic sterilization of liquids is described, and the author concludes from some experiments made with beer that such a process will considerably increase its stability without detriment to its quality. A. A. D. C.

Continuous Mashing and Flash Conversion in the Distillery. E. D. UNGER (*Amer. Brewer*, 1948, 81, Sept., 21-23, 56 and 58). To overcome the disadvantages of traditional methods of batch mashing in the distillery, clean, efficient, rapid and continuous processes, capable of automatic control, are being developed. The milled grain is mixed with warm water and stillage in a small pre-cooker, where a temperature of 145° F. and p_H 5.2 are attained. This mash is pumped to continuous cooker U-tubes, where it is raised instantaneously to 350° C. by high pressure steam. The temperature is maintained for 60 seconds and the mash instantly cooled to 145° F. by discharging into a vacuum flash

chamber. Under these conditions, the grain starch is completely gelatinized without caramelization. Barley malt slurry is admixed with the converted mash by continuously pumping it into the pipeline carrying the converted mash from the vacuum chamber. Sixty to seventy *per cent* conversion of starch to maltose takes place in the pipeline in 2 minutes at 145° F. and is accompanied by minimal amylose destruction under these conditions, so that conversion can still proceed during the subsequent fermentation. The converted mash is cooled in paraflows to 80° F. and pumped to the fermenters. The process described deals with about 5,000 bushels of grain *per* day, but can cope with double that amount. C. R.

Water and Hop Cultivation. J. G. LAMBERT (*Rev. Int. Brass. et Malt.*, 1948, Jan.-Feb., 1). What is true concerning the importance of water to all plants, is doubly true in the case of hops. Belgian growers believe that hops suffer from lack of water one year in three. Water stimulates the production of roots bearing the absorptive root hairs in the arable upper soil layer, *i.e.*, in the layer which contains the bulk of the plant nutrient substances and to which added manures can penetrate. Two 7-year-old hop plants were dug up from the same row, one having grown in a dry and the other in a moist place. The former had been forced to push down deep in search of moisture, and no root branches bearing root hairs were present in the arable layer or even to a depth of 1 meter. By contrast, the moist plant had produced numerous absorptive ramifications in the arable layer not deeper than 50-60 cms. Hop growing is intensive and pays for artificial irrigation, both in quantity and quality of hops. For successful irrigation, well worked soils are essential: the smaller the soil particles, the more water-retaining pores are present and the more readily can water reach all particles of clay and humus which retain water by virtue of their colloidal nature. The surface crust of soil should be broken up and water prevented from running away on sloping land by banking up the soil in small barriers erected after each row. Manures must be spread out evenly and, if insoluble, finely divided. It is preferable to use soluble manures in dry localities since insoluble ones need the intervention of more water before they can be assimilated. C. R.

Investigations on Hop Storage. W. GOEDKOOP (*Brewers' Digest*, 1948, Sept., 50). The deterioration of quality in Zatec hops as a result of storage was judged from analysis by Wöllmer's method to determine α - and β -acids and resins. The conditions selected consisted in storage for one year (1) in a brewery hop

storage room at 0° C.; (2) in a refrigerator at 6-8° C.; (3) at room temperature. The changes observed, compared with hops before storage taken as 100, are summarized in the table and show an increase in β -acid + soft resins. The conclusion is drawn that to obtain the same bittering effect, 18 *per cent.* more hops must be used after storage at 0° C. and 39 *per cent.* more after storage at 6-8° C.

	July 1946.	Brew- ery 0° C.	Refrig- erator 6°-8° C.	Room Temp.
Total resins, <i>per cent.</i>	100	106	100	100
α - + β -acids + soft resins, <i>per cent.</i>	100	102	95	96
α -acid, <i>per cent.</i>	100	80	66	22
β -acid + soft resins, <i>per cent.</i>	100	117	116	149
Hard resin, <i>per cent.</i>	100	103	121	117
α + $1/9\beta$	100	85	72	38
α + $1/3\beta$	100	92	82	62

C. R.

The Lime-Zeolite Process. J. G. WALKER (*Chem. & Ind.*, 1948, No. 44, 695-697). Waters treated with lime are supersaturated with calcium carbonate, and possibly magnesium hydroxide, both of which give loose deposit in pipelines and scale in boilers. They also contain an undesirable slight excess of lime. The lime-soda process has the added disadvantage of leaving some sodium hydroxide and carbonate in the waters, and it does not give waters of zero hardness. Moreover, soda ash is comparatively expensive. In the lime-zeolite process the tendency to "after-precipitation" of calcium carbonate and magnesium hydroxide, and excess of lime, are removed from the lime-treated water by passing it through a calcium zeolite. If required, the permanent hardness can then be removed by passage through the usual sodium zeolite. The process in outline is as follows. The water is treated with slight excess of lime, added preferably as lime water, and, if necessary, a coagulant, and allowed to settle for 1 to 4 hours. The water, after treatment, should have: alkalinity to phenol phthalein, 40-50; alkalinity to methyl orange, 60-70; and temporary hardness, 60-70; all expressed as *p.p.m.* CaCO₃ (it will also contain, of course, all its permanent hardness). This water need not be filtered before passing through a pressure filter containing carbonaceous calcium zeolite. The quantity of zeolite required is estimated on the basis that 1 cu. ft. (about 50 lb.) will remove 6,000 grains of calcium carbonate if regenerated

with a mineral acid, or 3,000 grains if regenerated with carbon dioxide. The zeolite should have a depth of at least 3 ft., and the distance between the top of the bed and the distributor should be two-thirds of the bed depth. The rate of flow should not exceed 300 galls. *per sq. ft.* of bed area *per hour*. The effluent water should have: alkalinity to phenol phthalein, nil; alkalinity to methyl orange, 10-15; and temporary hardness 10-15, as *p.p.m.* CaCO_3 . The p_H should be 6.9-7.1. If the permanent hardness is removed by passing this water through a base-exchange unit, the final effluent should have: alkalinity to phenol phthalein, nil; alkalinity to methyl orange, 10-15 *p.p.m.* CaCO_3 ; total hardness, nil; free carbon dioxide 2 *p.p.m.*; and p_H 7.0-7.2. The same conditions apply to the design and use of the base-exchange unit as to the calcium zeolite unit. The calcium zeolite may be regenerated with sulphuric acid (up to 0.5 *per cent.*) or hydrochloric acid (up to 10 *per cent.*), and about 2.2 lb. of commercial hydrochloric acid will be needed *per cub. ft.* of zeolite. Alternatively, carbon dioxide may be used for regeneration if conditions are suitable. The base-exchange zeolite is regenerated with sodium chloride. A modified process enabling stabilization and base-exchange softening to be carried out by a single bed of carbonaceous zeolite is briefly described, and the author concludes with a discussion of the applications of the lime-zeolite process.

A. A. D. C.

Bacteriological Analysis of Water used in Breweries. R. BAETSLE (*Chim. et Industr.*, 1948, 60, 96). In the method of analysis put forward, the bacteria in the water are concentrated by adsorption on alumina, which is then used for the inoculation of a series of selective media, followed by incubation at 25° or 37° C. (77° or 98.6° F.). Liquid media are simpler, but solid media give more exact results. Moulds, yeasts and a variety of bacteria can be distinguished in this way.

A. A. D. C.

Determination of Dextrins in Worts and Beers. G. VAN ROEY (*Chim. et Industr.*, 1948, 60, 94). The difficulty of determining dextrins in worts and beers is due partly to their heterogeneous nature and partly to interference by a number of other substances present. Most of the methods used hitherto are not specific, but this is not the case in the enzymic method devised by the author, which does not, however, escape the liability of error due to the reducing power of dextrins. Results obtained by the enzymic method are lower than those given by the acid-conversion method.

A. A. D. C.

Determination of the Activity of β -Amylase. G. NOELTING and P. BERNFELD

(*Helv. chim. Acta*, 1948, 31, 286-290; through *Brit. Abstr.*, C, 1948, 203). Amylopectin (isolated from potato by the Schoch method) is acted upon by the amylase solution under test, and the maltose produced when heated to 100° C. with 3:5 dinitrosalicylic acid in alkaline solution yields a brown colour the depth of which is proportional to the enzyme activity. Since this method is also satisfactory for determining the activity of α -amylase it is essential to ensure the absence of the latter before the above procedure is used. This is carried out by detecting its action upon the residual dextrin from maize amylopectin by which means it is possible to detect 0.01 *per cent.* of α -amylase.

T. J. W.

A Direct Colorimetric Method for the Determination of Carbohydrates. G. H. BENHAM and J. S. DESPAUL (*Analyt. Chem.*, 1948, 20, 933-935). Previous investigators have applied the formation of molybdenum blue to the detection and determination of sugars and the authors have modified the procedure to include the use of the photoelectric colorimeter in the determination of glucose. Into each of a series of flasks 5 c.c. of 0.02M potassium di-hydrogen phosphate, 10 c.c. of 7.5 *per cent.* ammonium molybdate and amounts of the sugar under investigation ranging from 1 to 10 mgrm. were added. The flasks were heated in an autoclave at 100° C. for exactly 30 minutes and were then cooled immediately in ice water. The intensity of colour was determined in a spectrophotometer at a wave length of 650 $m\mu$ (red) using tubes 19 mm. in length. Under the above conditions all monosaccharides yield a blue colour but disaccharides and raffinose have little effect. The presence of sucrose in glucose solutions has no appreciable effect upon the results unless the sucrose-glucose ratio and the total amount of sugar are both high. A comparison of the results obtained with such a mixture by using the above procedure were in excellent agreement with those yielded by the Munson and Walker gravimetric method.

T. J. W.

Elimination of Nitrite Interference in Iodimetric Sugar Determinations. J. CORMAN (*Analyt. Chem.*, 1948, 20, 984). Nitrites often occur in bacteriological media either being added as such or being produced by the action of micro-organisms and introduce serious errors in the iodimetric determination of any sugars present. This difficulty may be eliminated by adding to 10 c.c. of the medium 10 c.c. of 10 *per cent.* urea solution and 5 c.c. of N sulphuric acid. After standing 30 minutes with occasional shaking phenolphthalein indicator is added, the liquid neutralized with

sodium hydroxide solution and diluted to 100 c.c. A series of tests has shown that this procedure completely eliminates nitrites and is without effect upon glucose or maltose, but sucrose is hydrolyzed to a negligible extent.

T. J. W.

Determination of Malt Syrup Density.

R. H. FOSNOT and R. W. HAMAN (*Analyt. Chem.*, 1948, **20**, 955-957). The methods usually employed for the determination of sugar syrup densities do not combine the modern requirements of both speed and accuracy and the authors have investigated the correlation between density and viscosity since the latter may be determined easily. The instrument selected for the purpose was the Brabender viscometer used in the paint industry. The sample is contained in a circular vessel which is fixed on a platform rotating at a constant speed. A spring-controlled paddle is immersed in the sample and when the container is rotated a torque is imparted to the paddle, the movement and position of which is conveyed to a lever arm and an inking mechanism thus producing a permanent record. The apparatus may be adapted for use over a wide range of viscosities by altering the speed of the rotating platform, using springs of different strength and changing the shape and size of the paddle. By experiments carried out with syrups of different densities and at different temperatures followed by a mathematical analysis of the data obtained, the relation between the viscosity and the density was determined and a chart was drawn including the three factors. It was found that different consistency-density-temperature relations were shown by different types of syrup so that separate curves were determined for each. Comparative tests made on a series of syrups of different types by both the specific gravity and viscosity method showed the maximum difference to be $\pm 0.2^\circ$ Baumé. The method described necessitates no weighing, no dilution of the syrup, no adjustment to standard temperature and little operative skill, whilst it is much more rapid than other procedures and produces a permanent record for future reference.

T. J. W.

II.—FERMENTATION

Yeast Flocculation. Y. DE HEMPTINNE (*Chim. et Industr.*, 1948, **60**, 95-96). The flocculation phenomena here described are observed only under strictly defined conditions: a very dilute medium, heavy seeding rate and prolific growth. In these circumstances flocculation is preceded by the formation of elongated yeast cells of mycelial type, which form nuclei for

the clumping of normal cells. The formation of these elongated cells coincides with the disappearance of fermentable sugars from the medium, which suggests that at least one factor in flocculation is lack of these sugars. The elongated cells regain their normal shape after a few fermentations in stronger media.

A. A. D. C.

Fermentation of Disaccharides. I.—Reducing Disaccharides and Trehalose.

S. HESTRIN (*Wallerstein Lab. Commun.*, 1948, **11**, 193-207). Disaccharide fermentation involves a splitting of the linkage between the sugar units and the taking up of a molecule of water; according to the indirect fermentation theory of Fischer this involved the intervention of disaccharases, yeasts which failed to ferment a particular disaccharide being deficient in the corresponding enzyme. Doubt, however, was cast on the inevitability of such a mechanism by the work of Willstätter and others, but whilst the experimental findings of Willstätter are not open to doubt, the "disaccharidozymase" systems which he postulated to give direct fermentation are not so well founded. In the case of maltose, it has been found that the rate of hydrolysis by yeast treated with cytolyzing agents to suppress fermentation—whilst presumably leaving the maltase intact—is for many yeasts much slower than the rate of fermentation of this sugar by intact cells. Again, yeasts rich in maltase (*carlsbergensis* type) ferment α -methyl glucoside readily whilst some *cerevisiae* yeasts which are poor in maltase ferment this glucoside little if at all; yet both types ferment maltose readily. Also, acid treatment of yeast (which impairs maltase activity) has little influence on fermentation, whilst at 4°C . α -methyl glucoside is not fermented at all by yeasts which still actively ferment maltose under the same conditions. A similar conclusion that the intervention of maltase is not essential is reached from a consideration of p_H relationships of the two reactions. It is well known that glucosidic linkages can be split by phosphorolysis, under the influence of phosphorylase. If maltose underwent such action, the products would be glucose and glucose-1-phosphate; however, if this occurs, the necessary enzyme has yet to be discovered, since the known phosphorylases (including that of yeast) only attack polymers above a certain limiting size. An alternative suggestion is that maltose undergoes "polymeric cleavage," yielding a molecule of glucose with the second "glucosyl residue" attaching itself to a "glucosyl acceptor," thereafter being split off by phosphorylase in presence of phosphate to give glucose-1-phosphate. Maltase might function additionally, giving two molecules of glucose. The glucose, however

formed, would be converted to glucose-6-phosphate as in the normal fermentation of this sugar, whilst glucose-1-phosphate would be converted to glucose-6-phosphate under the influence of mutase. If several paths were thus available for maltose fermentation, it might be possible to explain the anomalous genetic behaviour with regard to fermentation which is sometimes observed. In yeasts which require adaptation to maltose fermentation, the mechanism by which the active enzyme system is produced still remains obscure, but it should not be assumed that complete synthesis of a new enzyme is necessarily involved.

Yeasts which ferment lactose contain lactase, but again fermentation can proceed under conditions which appear to preclude lactase activity, and it is frequently true that lactose ferments more rapidly than either of its hexose components. Whether yeasts can ferment melibiose by means other than after preliminary hydrolysis by melibiase is not known, but in the case of trehalose it seems probable that there is an alternative route which does not involve preliminary hydrolysis to glucose.

I. A. P.

Yield of Yeast in Yeast Manufacture.

M. H. VAN LAER (*Chim. et Industr.*, 1948, 60, 94-95). Yeast uses for its synthesis of cell substance the intermediate products of alcoholic fermentation, but the latter stage normally proceeds so much more quickly than does the formation of the intermediate products that fermentation products accumulate in the wort at the expense of yield of yeast. The most certain way of preventing this is to feed the growing yeast with only very small quantities of sugar at a time. In this way the intermediate products are synthesized into yeast-cell substance as soon as they are formed. Regulation of the rate of addition of wort to the yeast is, therefore, controlled by the activity of the enzymes concerned in this synthesis.

A. A. D. C.

Study of Yeasts by Simple Dialysis in Presence of Electrolytes and by Electrodialysis. G. HATTA and H. LECOQ. (*Chim. et Industr.*, 1948, 60, 95). Earlier work by the authors has demonstrated the apparent action of nickel salts on the permeability of the yeast cell membrane. In this continuation of their investigations they have dialyzed various yeasts and micro-organisms in the presence of certain electrolytes, and have carried their experiments with electrodialysis further by varying the metal used for the electrodes.

A. A. D. C.

Formation of Flavour Substances in Fermentation. H. LUERS (*Brewers' Digest*, 1948, Sept., 45-49 and 53). The type and com-

position of the raw material determines the flavour and aroma of fermented beverages. Because flavouring and aromatic substances occur in minute amounts in beer and wine, the work in this field is largely speculative. The author reviews the factual data available.

Aroma and flavour may be developed during yeast fermentation as a result of five types of reaction: (1) deamination of amino acids to yield the alcohols which constitute fusel oil (this *Journ.*, 1933, 597; 608); (2) hydrogenation of other hydrogen acceptors than acetaldehyde; (3) acyloin synthesis of carbinols of pronounced odour, according to the reaction: $R \cdot CHO + CH_3 \cdot CHO \rightarrow R \cdot CHOH \cdot CO \cdot CH_3$; (4) Cannizzaro reactions involving both acetaldehyde and fermentation intermediates: $2R \cdot CH_2CHO + H_2O \rightarrow R \cdot COOH + R \cdot CH_2OH$. In this way, higher acids may be formed whose esters have marked qualities of flavour and aroma; (5) formation of acids as yeast utilizes the ammonium ion from inorganic and organic ammonium salts, and also of those acids, like succinic, arising as a result of sugar assimilation.

Fusel oil is formed during the later stages of fermentation and is independent of yeast quantity. There appears to be a correlation between total acidity, ester and fusel oil during fermentation. Increase of fermentation temperature brings about a relatively greater increase in fusel oil than total acidity or esters. Malting with the aim of a high quantity of degraded protein is associated with higher ester, volatile acid and alcohol contents of the beers. Similarly, mashing processes (*e.g.*, acidified mashes) designed to give more complete protein breakdown, are associated with beers with a high ester content. In contrast to top and bottom yeasts, wine and wild yeasts produce considerable quantities of esters. Carlsberg and Froberg bottom yeasts yield little volatile acid, but top yeast (Genossenschaft), wine and wild yeasts yield more. The amount of by-product formed differs markedly, both according to the race of yeast and also to the condition of the yeast. Prolonged use of a yeast, or weak, degenerate yeasts, are often associated with high ester and fusel oil contents.

Maturation involves the removal of coarse and undesirable flavours as well as the positive development of substances like esters and higher alcohols which are intimately associated with the vital activity of the yeast. For 7-10 weeks storage, esters increase at the expense of total acidity. High storage temperatures favour the development of flavour, 4 days at 20° C. yielding as much fusel oil as is obtained in 3 weeks at ordinary storage temperatures. Lower bunging pressures give better flavours than high ones, probably because the former favour the removal of volatile harsh constituents. The increased amounts of higher alcohols and esters arising

during secondary fermentation are produced from amino acids, a fact which gives added significance to the nature of the original barley proteins. The removal of disagreeable flavouring substances from freshly fermented beverages is attributable to the volatilization of sulphur compounds (H_2S , mercaptans), aldehydes and acetals, in the CO_2 evolved during secondary fermentation. Thus, a stream of CO_2 , or degassing *in vacuo*, will free a "green" beer from these substances, the principle being employed in the Nathan quick maturation system. Maturation also involves adsorption-catalytic and surface-active reactions taking place on yeast cells, colloidal particles, chips, CO_2 bubbles and the surfaces of storage vessels. Beer matures more rapidly in small bulk, where the surface/volume ratio is relatively large.

C. R.

Acclimatization of Various Yeasts to Wood Sugar. M. C. JOHNSON and E. E. HARRIS (*J. Amer. chem. Soc.*, 1948, **70**, 2961-2963). Wood hydrolyzates are used as a source of sugar for the production of a high protein yeast and of alcohol but since inhibiting substances are present these tend to retard yeast activity. The authors have investigated the acclimatization of 21 different yeasts including 8 strains of *Torula utilis* to cultivation in the hydrolyzate of Douglas fir wood. This medium is produced by treating the wood under pressure with a current of dilute sulphuric acid, the resulting liquid being neutralized subsequently with lime and filtered. The liquid was diluted to contain 5 per cent. of wood sugar, the p_{H} value adjusted to 5.4 or 5.8 and small amounts of urea and potassium dihydrogen phosphate were added as nutrients. To the liquid, 1 per cent. by volume of the wet yeast was added and the temperature was maintained at 30° C. with continuous swirling. At daily intervals the yeast was separated and transferred to a fresh portion of the medium, about 30 successive transfers being made. Considerable variation was shown by the different yeasts in the amount of sugar utilized and the quantities of yeast and alcohol produced at the first transfer, but in the later stages when acclimatization had occurred the sugar fermented by all the yeasts ranged from 78 to 94 per cent., whilst the yields of alcohol varied from 31 to 40 per cent. and of yeast 30 to 42 per cent.

T. J. W.

III.—BEER

Control of the Colloidal Stability of Beers. J. DE CLERCK (*Rev. Int. Brass. et Malt.*, 1948, Nos. 3-4, 35-38). Colloidal hazes in beer are due to certain of the more complex proteins which have become oxidized, or have become insoluble by interaction with

a trace of heavy metal or with tannin. One means of gaining information on the colloidal stability of a beer is, therefore, to find how much of the more complex proteins it contains and then to determine its state of oxidation, its liability to oxidation, its content of heavy metals and its content of tannin. The author reviews the methods that have been proposed by Lundin and Schroderheim (this *Journ.*, 1931, 574), Kolbach and Buse (*ibid.*, 1933, 562), Bishop (*ibid.*, 1944, 224) and Hartong (*ibid.*, 1937, 121) for determining the complex proteins, and suggests that a modification of Bishop's method, whereby the precipitate given with trichloroacetic acid would be measured nephelometrically, is worth investigation. Control of aeration of the beer is gained by three determinations: the amount of air in the space above the beer in bottle, the air dissolved in the beer, and the Indicator Time Test value. Reference is made to De Clerck's method for the first (*ibid.*, 1937, 350), the method of Rothchild and Stone for the second (*ibid.*, 1938, 425) and that of Gray and Stone for the third (*ibid.*, 1939, 253). Heavy metals (iron, copper, tin, nickel, etc.) in the beer should not exceed 1 p.p.m. The author has recently published a method of determining tannin in beer (*ibid.*, 1947, 315). With all this information, inference of the colloidal stability is still uncertain because of the intervention of lesser known factors, and a far simpler test is to keep samples of the beer at 40° C. (104° F.) or even 60° C. (140° F.) and measure the chill haze at intervals of, say, 2 days. If this test shows that the beer is unstable, then the cause of the instability—complex proteins, too much tannin, excessive aeration, or too high a contamination with heavy metals—can be investigated by the methods which have been cited above.

A. A. D. C.

Study of the Rapid Maturation of Beers. F. GAENG (*Chim. et Industr.*, 1948, **60**, 94). The author claims to have produced beers of fine quality by a process of rapid maturation involving controlled oxidation. Oxidation is considered to be the principal factor in normal maturation, and in addition to describing the artificial maturation process the author discusses the accurate measurement of the degree of aeration of a beer.

A. A. D. C.

Filtration with Kieselguhr. J. G. LAMBERT (*Pet. J. Brass.*, 1948, **56**, 814-819 and 831-833). As a filtering agent kieselguhr acts partly by adsorption of suspended particles and partly by mechanical retention at the filter surface. The finer the particles the more adsorption predominates, and *vice versa*. A kieselguhr beer-filter consists of two parts: a small tank to hold the slurry of kieselguhr (in beer or water), and a compartment enclosing a

set of filter elements. Each element consists of a frame on each side of which is stretched a woven wire sieve, preferably made of bronze or stainless steel. The beer passes from the outside to the middle of the element in course of filtration, which requires two operations. First, a layer of kieselguhr about 0.01 in. thick is deposited on the wire meshes by means of pumping the necessary amount of slurry through the filter. About 1 lb. kieselguhr *per* sq. yd. of filter surface is required; excess must be avoided, and the layer must be compact and even. This is achieved by making the layer under a pressure not less than that subsequently used for filtration, and by commencing filtration immediately afterwards without allowing the pressure to be released. For the second operation (the filtration proper) kieselguhr is injected in measured amount from the slurry tank into the beer at a point between the beer pump and the filter. An injection pump of the diaphragm type is best, and the amount of kieselguhr added should be equal to or slightly more than, the amount of suspended matter in the beer. This will form a cake of the right texture on the filter. Too little kieselguhr will give a cake lacking in porosity, which will retard filtration; too much is wasteful, fills up the working space of the filter more rapidly than is necessary, and may give too fine a filtration, to the detriment of the beer. The grade of kieselguhr (particle size) is even more important; too coarse a grade will not give a bright beer, while too fine a grade will rob the beer of character and fulness. To find the quantity of kieselguhr needed, some of the beer is centrifuged and the amount of matter deposited is measured. The correct grade of kieselguhr is determined for each type of beer by making experimental filtrations and determining the clarity and foaming power of the filtered beers. A grade giving adequate clarity with no appreciable loss of foaming power is satisfactory. This laboratory control is essential to efficient kieselguhr filtration. Given that, the kieselguhr filter is simple, robust, easy to dismantle and clean, requires little attention, and costs less to run than a pulp filter of the same output. The filtration loss is very low and filtration is very regular, the output being the same from start to finish. For beers which contain a lot of matter in suspension it is far superior to any other form of filter. A. A. D. C.

Air and Carbon Dioxide in Beer. B. D. HARTONG (*Rev. Int. Brass. et Malt.*, 1948, Nos. 3-4, 25-34). Beer is carbonated for two reasons: first, because it adds to the flavour of the beer—and the weaker the beer the more essential is proper carbonation—and secondly, because it aids head formation. The author outlines the four methods most often used for

determining carbon dioxide in beer: the gravimetric, in which the evolved gas is trapped in caustic potash and weighed; the semi-gravimetric, in which the gas is trapped in caustic potash which is titrated before and after; the titrimetric, in which an excess of alkali is added to a portion of the beer and the excess titrated; and the manometric, in which the gas content of the beer is related to the pressure exerted by the evolved gas. For routine work the author prefers the manometric method, with an occasional check against the titrimetric figure. From observations made over the past 10 years, it appears that head formation is linearly related to carbon dioxide content (regression coefficient, + 0.94) and that the most desirable head (1.2 in. in a 12 oz. glass) is achieved with a content of 0.42 *per cent.* Tests have shown that there is an average loss of 2 *per cent.* of the gas during racking into casks, and of 9 *per cent.* during bottling, so that beer intended for cask should contain (in tank) 0.43 *per cent.* and beer for bottling 0.46 *per cent.* of gas. A complication arises because the gas content of a beer in tank increases with increasing depth. In a 9 ft. depth, for example, the beer at the bottom has been found to contain 0.07 *per cent.* more carbon dioxide than the beer at the top. If fermentation is still going on this gradient will, of course, be less defined owing to movement of the beer. Care should be taken that the gas used for carbonation contains no air and no traces of surface-active substances.

Air has a necessary part to play in brewing up to the stage of fermentation. Beyond this point it is not wanted, except possibly for a small amount which may assist maturation, and this is particularly true for bottled beer. Beer in tank need never contain more than 1 to 2 c.c. of air *per* litre, but some air is trapped in the space above the beer when it is bottled. This air may be determined in several ways, all of which operate on the principle of removing the carbon dioxide from the total gas in the bottle and measuring the volume of the residual gas. The oxygen in this air is slowly absorbed by the beer, and further information is given by a determination of the r_H of the beer, which indicates how far oxidation of the beer has proceeded. The third item of information available is the concentration of oxidizable substances in the beer, and this is most readily expressed as the Indicator Time Test value in which the time taken for the beer to achieve 80 *per cent.* decolorization of a redox indicator is measured. The author has found it an advantage to take as the end point sometimes less than this degree of decolorization, sometimes total decolorization, according to the type of beer being examined. It is essential to make all measurements of I.T.T. value at

the same temperature because of the large variation in the value with temperature change. From I.T.T. values determined on a large number of beers of different gravities during the past 10 years, the author has found that there is a linear relationship between the original gravity of the beer and the logarithm of the I.T.T. value computed to total decolorization, the correlation coefficient being -1.0 . Since in all these tests care was taken to keep the beer out of contact with air, it is considered that the results show the concentration of oxidizable substances in a beer to be determined solely by the original gravity. To keep a control over the aeration of a beer all that is necessary, therefore, is to determine its I.T.T. value and see that it does not exceed the figure found to correspond to the original gravity of the beer. The principal oxidizable compounds in beer are the reductones, and the author has found by experiment that these compounds are similar in their behaviour as oxygen carriers to ascorbic acid. Moreover, beer contains sulphhydryl groups combined with its nitrogenous components and these will act as oxygen acceptors as they do for ascorbic acid. The ill effects of aeration on beer are summarized as (1) increased liability to chill (oxidation) haze, resulting from oxidation of tannins and, probably, certain sulphur-bearing protein derivatives; (2) development of a reddish tinge, in which the free tannins appear to be concerned; (3) changes in aroma and flavour, especially as a result of pasteurization; and (4) loss of bitterness owing to oxidation of hop resins. The pasteurization aroma and flavour are due to volatile substances, possibly esters.

A. A. D. C.

Determination of the I.T.T. (Indicator Time Test) Value of Beer. M. DE RIJCK (*Pet. J. Brass.*, 1948, 56, 873-874). The Indicator Time Test was devised by Gray and Stone (this *Journ.*, 1939, 253), and its value is expressed as the time, in seconds, taken by 10 c.c. of the sample to bring about 80 per cent. decolorization of 0.25 c.c. of the specified indicator solution at 25° C. (77° F.). The latter is a redox indicator of the 2-6-dichlorophenol-indophenol type. Normal beers have an I.T.T. value of 100-150, but for strongly reducing beers the value may be as low as 50, and for beers with a weak reducing power it may reach 3,000. In the course of the brewing process the I.T.T. value is increased by the action of atmospheric oxygen, except during the copper boil when it is lowered by the entry into the wort of reducing substances from the hops and the formation of melanoidins. The test has three disadvantages: the indicator is very sensitive to temperature, the test is a subjective one, and the indicator is difficult to obtain. Its advantages are that it is easy

to perform and requires only simple apparatus, the indicator is practically immune to the action of atmospheric oxygen (in contrast with the sensitivity of the electrodes used in electrometric methods), and the test can be readily carried out at any stage of brewing (*cf. ibid.*, 1939, 443).

A. A. D. C.

IV.—PLANT, MACHINERY AND BY-PRODUCTS

Rapid Identification of Defects in Heat Exchangers. C. C. DOWNIE (*Brewers' J.*, 1948, 84, 502-504). Most methods used for the detection of defects in heat exchangers are more or less unsatisfactory for various reasons and often lead to the scrapping of tubes which would be suitable for further service. One of the newer methods was based upon the use of gamma rays from a radium source which operated with tubes of any metal, but this procedure was relatively expensive. An electronic type of apparatus has been devised which is applicable to exchanger tubes of non-ferrous metals and stainless steel. This will detect all kinds of defects including erosion and corrosion pits, cracks, pinholes, changes in wall thickness and variations in composition. The equipment comprises one or more interchangeable probes for insertion into the tubes, a device for pulling the probes through the tubes which is synchronized with an electronic recorder provided with a continuous strip chart and a neon lamp which gives a visual indication of any defect in addition to the permanent record. The head of the exchanger is removed and the probe is drawn through a tube at a definite rate and any defect and its location is automatically indicated on the chart. The results are unaffected by the presence of scale or any other non-metallic deposit. Further research is in progress to render this electronic "sensing" procedure adaptable to tubes of iron and steel. T. J. W.

Mechanical Turners in Pneumatic Malting. G. KAUERT (*Chim. et Industr.*, 1948, 60, 94). A discussion of the relative merits of mechanical turners of the plate and screw types. Under the conditions general in Belgium, where 1 or 2 turners have to do service for 8-10 malting cases, the author considers that the plate type is more economical, more efficient, and easier to use than the screw type.

A. A. D. C.

V.—BIOCHEMISTRY

Structure of Starch. K. MYRBÄCK (*Wallerstein Lab. Commun.*, 1948, 11, 209-219). The introduction within recent years of new methods for the fractionation of starch has allowed it to be shown that two components are present, amylose (usually amounting to some 25 per cent. of the starch substance) and

amylopectin. The viscosity of amylose solutions indicates the possession of unbranched chains of glucose residues, the degree of polymerization being of the order of 200-300 glucose units; the results of end-group assay are in accord with this. Amylopectin, on the other hand, gives an end-group value of 5-6 *per cent.*, and this—in view of the extremely high molecular weight—can only be accounted for on the assumption that the molecule is very highly branched. General agreement on the mode of branching has not been reached, but extreme possibilities are represented by the laminated formula of Haworth, Hirst and Isherwood, and the ramifying formula of Meyer.

It is a conspicuous feature of the action of amylases on starch that conversion into fermentable sugars is seldom if ever complete, limit or stable dextrans resistant to further amylolysis and to fermentation being formed. The facts can best be explained on the hypothesis that the amylases attack only 1·4- α -glucosidic linkages, which account for more than 90 *per cent.* of the linkages in the starch molecule and represent the normal linkages in the glucose chains. At intervals in the chains, however, other "anomalous" linkages occur, *e.g.*, at points of branching or where phosphoric acid is substituted; the amylases have little or no action on such anomalous linkages, and portions of the starch substance in which these persist after amylase action constitute the limit or stable dextrans. β -amylase of malt is an exo-amylase, the action of which is confined to splitting off maltose from the non-reducing ends of normal chains; it produces substantially 100 *per cent.* of maltose from amylose, but only about 50 *per cent.* of amylopectin is saccharified, since the branched portion cannot be penetrated and remains as β -dextrin (amylodextrin). α -Amylase is an endo-amylase which has high affinity

for long chains, to which it attaches itself at points remote from end-groups and anomalous linkages; the affinity of this enzyme for chains of less than 8 glucose units is low. Thus, "normal" α -dextrans are produced from those parts of the starch which are attackable by β -amylase, and "anomalous" α -dextrans from the branching portion of the amylopectin. These anomalous dextrans on further degradation by either amylase can only be incompletely saccharified because of the presence of the anomalous linkages; hence, the stable dextrans result from the original points of branching. Similar considerations apply to the amylolysis of glycogen, which resembles amylopectin in structure apart from possessing a higher degree of ramification. I. A. P.

Reversible Action of *B. macerans* Amylase. D. FRENCH, J. PAZUR, M. L. LEVINE and E. NORBERG (*J. Amer. chem. Soc.*, 1948, **70**, 3145). Since the conversion of starch to a cyclic Schardinger dextrin would probably have a low energy increment it is likely that the reaction should be readily reversible. The authors have investigated this possibility by treating a mixed solution of cyclohexa-amylose and maltose with the amylase from *B. macerans* when the polarimetric rotation was found to increase. Isolation of the products by fractional precipitation yielded a syrupy liquid having an $[\alpha]_D + 163^\circ$ and an average chain length of 8·9 glucose units. On the addition of iodine solution the colour was slightly darkened and the Tilden test failed to show the presence of any cyclohexa-amylose. This fraction, on being treated with *B. macerans* amylase, was rapidly converted into cyclohexa-amylose. Similar results have been obtained by substituting other sugars for the maltose. Thus the amylase from the above organism exhibits a synthetic as well as a degradative action.

T. J. W.

REVIEWS

BRITISH INTELLIGENCE OBJECTIVES SUB-COMMITTEE. Final Report No. 1512. Item No. 22. *German Brewing Industry*. By J. H. PORTER, A. CLARK DOULL, J. TODD, L. R. BISHOP, G. OSGOOD and F. E. LORENZ. London: H.M. Stationery Office, 1948, price 14s. net.

At the termination of hostilities in 1945 the Board of Trade promoted a series of investigations into German industrial, technical and scientific processes, of interest and advantage to this country. As part of this scheme the Brewers' Society, under the sponsorship of the Ministry of Food, invited a team composed of

experts representative of the principal sections of the brewing industry to visit Germany for the purpose of procuring information of general interest and value to the industry. The information acquired has been published in B.I.O.S. Report, No. 1512 (H.M. Stationery Office) and described under the heading of the targets visited. The present Report is a re-classification of that information with the addition of such matters from other official sources which are of interest to the brewing industry.

This Report, now under review, is an important document rich in informative matter.

Readers will find it of most compelling interest, whether in the methods of procedure, figures of production, details of plant, or the movement to develop non-alcoholic drinks and very low gravity beers.

Even the most casual reader cannot fail to pause momentarily at some item which must inevitably attract his attention, however quickly the pages are turned over. It must not be thought, however, that any fundamental knowledge connected with plant lay-out or with the actual process has been brought to light, because, as the authors state, the brewing process traditionally associated with continental beers is followed everywhere. The main interest lies in the description of details of plant and the treatment at the various stages of the malting and brewing processes where they are similar to those of this country. The survey indeed serves to emphasize further the point that there is no "secret" process in brewing. The real secret of brewing (in the reviewer's opinion) is to be found in combining the brewer's own practical and scientific knowledge and in his method of applying that knowledge to suit the circumstances of his own brewery and that of its trade.

The tour, for practical reasons, was confined to the American and British zones. Visits were made to 9 breweries, 4 maltings, 4 research stations, 4 consultants and other firms connected with brewing. The information was acquired by direct approach and by questioning, where possible, the heads of the concerns and departments. Naturally in the confines of a brief review it is possible to touch on only the merest fraction of the multifarious topics reported on. In the 9 breweries visited there is much that is common to all, but the views expressed differed widely in the approach to many subjects, *e.g.*, the most suitable material for the construction of fermentation vessels, or the best method of protein stabilization. There is a unanimous desire for wooden casks, but owing to the scarcity of timber most breweries have been compelled to use containers of stainless steel, aluminium, plain steel coated with a vitreous finish and laminated wood. None of these vessels gave complete satisfaction.

The methods employed by Beck in making their famous export beer are of considerable interest, and it is significant of German methods that this firm was subsidised by the Government to the extent of 30 *per cent.*, otherwise their competition in the export market after 1931 would not have been so effective.

Malting procedure is freely discussed and the balance between hand-made floor malt and box and drum-made malt is about even. Poor barley is best malted on the floor—but with good quality grain all methods yield sound malts. The appearance of the cement-surfaced

malting floors attracted considerable interest among the party. The floors are exceptionally hard and possess a smooth marble-like finish of great lasting quality. Details of the construction are given. To sum up, the information provides a good over-all picture of German pre-war malting and brewing technique, the difficulties and problems of the war period and the troubles and improvisations of the post-war era.

Little sympathy for the Germans will be aroused by the story of low gravity beers. The trend in the reduction of gravities, chiefly directed against the alcoholic content of beer, was started years before the war under the Nazi regime, influenced no doubt by the teetotalism of Hitler and various other causes. During the war, gravities were lowered still further and continued to a very extreme degree during the post-war food shortage. Beers with original gravities between 1,008° and 1,003° were permitted at the time of the visit. Great interest is attached to the special technical and biological problems associated with the development of these weak beers and much of the time of the research institutes has been spent on solving these problems. Concomitant with the production of this class of beer is the problem of infection, whereby different kinds of organisms become prominent as sources of infection as the gravity is lowered. Other interesting and surprising points were brought to light, *e.g.*, weak beers are more liable to infection than the corresponding worts. It was also found that boiled water used for dilution is more sensitive to infection than raw water. A natural step from low gravity beers is the production of non-alcoholic drinks, and this has met with varying degrees of success. Professor Schnegg considered that the best method of producing non-alcoholic (0.5 *per cent.* by weight alcohol) beer was along methods of normal brewing procedure and his method is described in a chapter in the appendix.

The appendix is further enriched by the translation of an abstract of a full report on the "Use of Forced Malt" by H. Fink, and by the description of a "Process for Cleaning and Sterilizing of Filter Pulp," by Dr. Pauly. There is also a schedule of Staff of the *Versuchs- und Lehranstalt für Brauerei*, Berlin, showing their war-time and present activities. The extract translated from the Statutes of the Experimental and Teaching Institute for Brewing in Berlin will be useful for purposes of comparison to those interested in the conditions governing membership of the Institute of Brewing.

Criticism might be levelled at the paper covers, the style of printing and the lack of index. Those interested in the brewing industry however, who read this document, cannot fail

to gain from it new ideas, either directly or by suggestion, which would immeasurably compensate for the small outlay of 14s.

L. FLETCHER.

Lancashire Boiler and Economizer: Defects and Repairs.—By SYDNEY D. SCORER, A.M.I.Mech.E., M.I.Mar.E. John D. Troup, Ltd., 90, High Holborn, W.C.1, 1948. Price 6s.

The author has appreciated the need of the plant engineer responsible for the operation and maintenance of shell boilers for some guidance on the data and reasons underlying the findings and recommendations of the boiler surveyor on boiler failures and repairs. Formula for the strength of boiler shells, furnaces and end plates is given, and its practical application to specific problems of failures and repairs, clearly explained. Furthermore, useful information on how certain maintenance work should be carried out in practice is also provided. The effects of corrosion, both external and internal, on boiler furnaces, flues and economizers—which is one of the most common troubles in shell boilers—is dealt with in some detail, and the importance stressed of careful inspection and testing where and when corrosion is suspected.

The cause and effect of failures in shell boilers is covered in a comprehensive and practical manner, and the importance of periodical examinations apart from those called for under the Factories Act, together with the necessity of maintaining correct feed water conditions, is emphasized.

The author rightly points out the danger of boiler operators allowing low water conditions to develop, and stresses the fact that by far the largest number of furnace collapses are due to this condition.

Some useful information is given on the fitting of patches to boiler shells and the importance of properly proportioned D-patches and the method of calculating the correct size is explained. The *raison d'être* of the D-patch is that the seam securing it to the boiler shell is a form of helix whereby a seam which, through corrosion, has become weakened, can be made equivalent to a longitudinal seam with a relatively high efficiency.

The use of welding in boiler repair work is not dealt with in as much detail as one would have wished, as it is in such general use to-day.

The notes on economizer operation and repair should serve as a useful guide to the maintenance engineer, and in these days of fuel economy and scarcity of plant, it is pointed out that an old economizer can still do useful work as a simple water heater when arranged on the suction side of the feed pump, thus avoiding pressure conditions in the economizer. The

effect of graphitic corrosion on economizer tubes is always difficult to detect by the ordinary visual methods, and the author gives some useful advice on how it can be located in the more or less inaccessible sections of a bank of economizer tubes.

The dangers of "steaming" in an ordinary economizer used for water heating only is, as is generally known, one of the most dangerous conditions that can arise during operation. This trouble frequently develops in the economizer in such factories as breweries where large "draws" of steam are followed by correspondingly heavy returns of hot water to the hot well.

This condition of steaming is caused by hot gases passing through the economizer with the feed pump stopped, a state of affairs which should not be allowed to develop if all precautions are taken, *i.e.*, when the feed pump is stopped, the economizer by-pass dampers should be opened and the main dampers closed.

M. W. PLUMPTON.

REPORTS ON THE PROGRESS OF APPLIED CHEMISTRY. 1947. Published by the Society of Chemical Industry. *The Fermentation Industries.* By E. C. BARTON-WRIGHT and E. R. DAWSON. Price 25s.

The Applied Chemistry Reports for 1947 comes out in slightly altered format. The volume is obviously smaller than its predecessors, but this is due to the use of thinner paper and to a closer setting of the type. References are numbered and appear at the end of each article and not as footnotes, but the monographs have not been appreciably curtailed and the various branches of applied chemistry are all included as before and each subject dealt with by experts who are intimately concerned with the industry on which they write.

Economy of print and paper is, of course, essential, but easy reading and reference should be secured where possible. Throughout this book, as also in all Chemical Society publications, consecutive references to the same journal are designated "*ibid.*" The extra print involved in restating the source would be very little, it would, in our opinion, make for easier reading, and the possibility of error would be greatly reduced.

The report with which we are most intimately concerned, that on the Fermentation Industries, is by E. C. Barton-Wright and E. R. Dawson. In this, as in other reports, a slight difference in treatment is introduced this year in that reviewers have selected aspects of their subject for fuller discussion and omitted merely cursory mention of the many other publications which have appeared during the year.

In the report on the Fermentation Industries variations in the hop and barley crops are

discussed and progress is reported in the search for disease resistant hops. Attention is directed to the work on propagation trials which will provide a means of rapidly increasing the commercial cultivation of selected varieties.

Bishop's work on the dormancy of barley is discussed, but we are rather surprised to find that the reference is to a paper in *Chemistry and Industry* and that no mention is made of the very much fuller report on the subject (this *Journ.*, 1947, 86).

The growth and separation of yeast in fermenting wort under bottom fermentation conditions has been studied very fully by Helm and Trolle and the reviewers describe the work in sufficient detail to show the methods used by the authors and which might be applied in any brewery to study the mechanism of the fermentation process.

Studies on the acetic bacteria by Walker and his colleagues are reviewed but no mention is made of their paper on the "Oxidation of Glycerol by *Acetobacter Species*" (this *Journ.*, 1947, 258) which might well have been included in this year's report. The section on microbiology concludes with a summary of work on the incidence of ropiness in beer and, taking this section as a whole, we can see that some very real progress has been made during the year in the bacteriology of brewing.

Improvements in penicillin production take an important place in the section on industrial fermentation, but the older fermentation processes are still developing and it is rather interesting to see that American agricultural authorities are studying the possibility of farm-scale production of power alcohol combined with utilization of the residues as an enriched protein stock feed.

It is easy to find omissions in an Annual Report, but the authors obviously cannot discuss every paper which has been published, and so we must allow them the right to make their own selection. They have given us an excellent survey of progress in the fermentation industries and we are grateful to them for presenting the fruits of an extensive study of many original papers in such eminently concise and readable form.

We cannot discuss the reports on other industries contained in this book, but we would commend them to the attention of our readers for, as users of their products, brewers are concerned with the progress of many industries besides their own. The volume of published research seems to increase each year; no one can hope to read it all in detail, so what can we do better than study the report of an expert on the progress of his own industry?

In conclusion we would commend the Annual Reports that they are issued bound to subscribers in handy octavo volumes. They might almost pass as bed-side books; certainly they can be collected in a bookcase of

normal dimensions to provide a reference library that is at once up to date and of unquestionable authority.

J. A. BURNS.

Diary for the Brewing and Syrup Rooms (1949).
A. Boake, Roberts & Co., Ltd., London,
E.15.

Many of those engaged in the brewing and soft drinks industries will welcome the issue of this publication, the forerunners of which, for some 57 years, have proved of considerable utility in many places. It follows the same general lines as its predecessors, but the section devoted to soft drink manufacture occupies twice the number of pages given to the brewing and excise tables.

The text opens with a continuation of the well-known series "Who's Who in the Brewing Room," in which brief biographies of six present-day brewers are given. In "Change and Decay," by A. J. C. Cosbie, the author discourses in his erudite and pleasant characteristic style on the preservation of foods, etc., and the outstanding historical developments in the use of antiseptics of which so much is based upon the researches of Lister and Pasteur.

To anyone interested in delving into the past "The Porter Brewery" will appeal strongly. This is an extract from a guide to the City of London, published in 1802 and provides an account of the origin of porter and a description of Messrs. Whitbread & Co.'s brewery as it was nearly 150 years ago. The title of the last article "Concentration and After," appears somewhat enigmatic until a perusal shows that it deals with the concentration of soft drinks during the war in order to economize bottles, labour and transport. The Order of 1943 was revoked early this year and the author, K. Penn, gives an account of the developments which have taken place since then and the possibilities of the future, whilst incidentally illustrating the wasteful obstinacy of a Government department when dealing with industrial matters.

These original articles are followed by numerous tables, etc., of value in the brewing and syrup rooms after which over 100 pages comprise the diary and memoranda. On pages 75 and 76 calendars and first aid instructions are provided, but the reviewer would suggest that the latter should be inserted in the extreme front of the volume in order to be more readily accessible in cases of emergency.

The data given in the numerous tables show a high degree of accuracy, but it may be indicated that several page numbers are missing, and a 2 is omitted from the formula for carbon dioxide on page 45.

The publishers deserve commendation for their enterprise in issuing this volume free of charge as it will prove a valuable addition to the library of the brewer and others engaged in beverage manufacture.

T. J. WARD.

JOURNAL OF THE INSTITUTE OF BREWING

MONTHLY REVIEW

EUROPEAN BREWERY CONVENTION. LUCERNE CONGRESS

Further details of the Congress to be held in Switzerland at Lucerne from 29th May to 3rd June, 1949, are now available. The principal papers to be discussed are under two headings:—

(a) *Proteins in Brewing*:—Contributors will be H. Lundin (Sweden), General Report; E. C. Barton-Wright (Great Britain); B. D. Hartong (Netherlands); J. H. St. Johnston (Great Britain); N. Kent and M. Macheboeuf (France); L. Massart (Belgium); E. Sandegren (Sweden); F. Tombeur and G. van Roey (Belgium) and B. Trolle (Denmark).

(b) *Production of Sterile Beer for Sale by Means other than Pasteurization*:—H. Kringstad (Norway), General Report; H. R. Jacobsen and J. Nestaas (Norway); A. Juillerat (Switzerland) and G. Osgood (Great Britain).

Further papers will be given by Messrs. P. P. Gray, S. Laufer and F. E. Siebel (U.S.A.), whilst a report will be presented by the Barley Committee on the state of barley culture in Western Europe, the following contributing:—H. van Veldhuizen (Netherlands), Chairman; T. Bendixen (Norway); L. R. Bishop (Great Britain); H. Thunaeus (Sweden) and E. Vestergaard (Denmark).

General information concerning the Congress has been given in a previous issue (this *Journ.*, 1949, 1).

* * * *

FERMENTATION INDUSTRIES SYMPOSIUM

ST. ANDREWS, 23-30TH JULY, 1949

Arrangements have now been announced for a Symposium sponsored by the Scottish Sections of the Royal Institute of Chemistry as part of a general programme of Scientific Courses, Conferences and Symposia. The

first Symposium in Scotland was held at St. Andrews in July, 1947, and was very successful in presenting a comprehensive survey of recent activities in the field of organic chemical industry. In response to a demand for further conferences on similar lines, arrangements have now been made to treat the subject "Recent Advances in the Fermentation Industries" under the following headings:—

Potable Fermentation Products: yeast; proteins and carbohydrates encountered in malting and brewing; comparison of British and Continental brewing methods.

Panary Fermentation.

Industrial Fermentation: (a) 2 : 3 butylene glycol; (b) glycerol; (c) gluconic acid; (d) *l*-sorbose; (e) acetone-butanol; (f) ethyl alcohol: methods of isolating the products, and aseptic technique in industrial fermentation. The use of fungal amylase in the industrial production of alcohol and alcohol products.

Mould Metabolic Products and Methods of Assay: including the assay of vitamins and amino-acids; micro-biological assay of penicillin, with special reference to micro-chromatographic technique.

Amongst the lecturers are some of the most prominent authorities on the subjects, including: E. C. Barton-Wright, E. V. Bell, J. M. Birkinshaw, L. R. Bishop, C. R. Bond, G. G. Freeman, E. Gill, E. Helm (Copenhagen), R. W. Jackson, D. W. Kent-Jones, A. Olsen, A. Parker and I. A. Preece,

It is intended to cover both the academic and industrial aspects of the subjects. Two lectures, each of one hour and followed by a short discussion, will take place each morning. A third lecture and discussion will be held in the early part of the evening. A detailed programme will be available later. The Biochemical Society and the Physiological

Society have agreed to co-operate, and will arrange for the presentation of papers, *etc.*, on Friday, 29th July (in Dundee), and on Saturday, 30th July (at St. Andrews).

Those attending the Symposium may be accommodated in students' residences and such accommodation will be available from the evening of Saturday, 23rd July (when visitors are advised to arrive) till the morning of 30th July. The fee for attendance is two guineas and for residence six guineas. A certain number of double rooms will be available for those accompanied by their wives; only one attendance fee will be required for a married couple. Those desirous of attending should apply to the Assistant Secretary, Royal Institute of Chemistry, 30, Russell Square, London, W.C.1, for an application form which should be completed and returned as soon as possible, together with the attendance fee. Priority will be given to visitors from overseas and to Fellows, Associates and Registered students of the Royal Institute who make application before 3rd May. After that date applicants will be notified of allotment of places.

* * * *

BREWING RESEARCH IN U.S.A.

Particular interest will be felt at this time in the establishment by the Master Brewers Association of America of a Research Foundation, the organization and objects of which are outlined in the *Communications* of the M.B.A.A. for Nov.-Dec., 1948. The scheme may be said to owe its inception to the establishment in 1940 of a Protein Research Fellowship at Stanford University, followed by the formation in 1945 of a Research Foundation Committee, as a result of whose deliberations the by-laws of the M.B.A.A. have now been amended to bring the Foundation into being. The reported remarks of Mr. J. G. Shakman, explaining the need for the scheme, are worthy of close attention. Pointing out that many organizations within, and related to, the brewing industry are carrying out research, Mr. Shakman noted the handicap under which much of the work is being done as a result of expense, duplication of effort, and lack of sufficient contact with the practical side of the industry; it was thus desirable to have co-ordination of effort on an industry-wide basis, the proposed scheme being organized by the M.B.A.A. (representing the professional brewer), with such

practical results as become available being made known to all. Further, for the scheme to be successful, not only must full financial assistance for the project be forthcoming, but it must also have full moral support.

Further details of the scheme were outlined by other speakers to a Convention of the Association. A Projects Committee, working under the Board of Trustees, would suggest subjects for investigation and would examine suggestions presented to it; suitable workers and institutions would be nominated to investigate each approved topic, and the work would be carried out under the direction and supervision of a sub-committee. Such a scheme would ensure flexibility, as it could begin on a small scale and later be expanded as the need arose. Results would be available to all members, and it was proposed to present reports in a readily understandable form, so that in appropriate cases they could be immediately applied to the brewing process.

The aims of the Foundation will meet with nothing but sympathy among members of the Brewing Profession everywhere, and it is hoped that a valuable measure of success will be achieved. That there is a tremendous potential field for brewing research cannot be denied, probably made more extensive by the different brewing techniques followed in different countries. The whole position may be summed up by quoting a sentence from the M.B.A.A. *Communications* report: "To try to obtain maximum results from the knowledge available from each branch of science is hopeless, unless under co-ordinated direction, and this is the basis of industrial research."

* * * *

BARLEY VARIETIES

At a time when much is heard of barley varieties, many people will be glad to have an authoritative botanical description of types regarded as suitable for growth in this country, to supplement their views on the characters of these varieties from the malting and brewing points of view. Such a description is provided in Dr. F. Earnshaw's *Description of the Recommended Varieties of Wheat and Barley*, published by the National Institute of Agricultural Botany. The text pages devoted to barley are considerably fewer in number than those given to wheat,

and it is pointed out that the limited number of barley varieties (the *Recommended List* contains only ten) arises from the provision of new varieties by breeding and from the fact that since the home-grown crop is intended primarily for malting the desired range of acceptable quality is limited; an exception is provided by Camton, introduced as a high-yielding variety for stock feed. Characters which have usually been sought in introducing new varieties include early maturity or winter hardiness and strength of straw. Prefect is a six-row barley bred from Praecox and Spratt Archer; it matures a few days earlier than commercial six-row and the straw stands better; although yields have been somewhat low, the grain is of superior quality with a plumper appearance than is usual with barleys of this type. Three varieties—Earl, Golden Archer and Pioneer—have been derived from Spratt Archer by selection or hybridization. Earl (a selection) matures 7–10 days earlier than the parent variety and may prove of value in districts where harvest is late for Spratt Archer and also in permitting a more convenient spread-over of the harvest of large barley acreages. Pioneer (Tschermak's Two-Row Winter $\times$ Spratt Archer) appears to be of good malting quality; it has a higher degree of winter-hardiness than Spratt Archer, being suitable for autumn sowing. It has short straw and matures early. Plumage Archer 1943 has been derived from the cross Spratt Archer 37/3 $\times$ Plumage Archer 1935 and matures a day or two later than Spratt Archer, than which it is better adapted to heavier and more fertile soils and to areas of greater rainfall. The Danish variety Kenia was first introduced into Britain about 1933 and frequently gives higher yields than the established varieties, the short stiff straw causing reduced lodging. It is significantly stated, however, that it is doubtful whether it is as widely adapted to conditions in this country; with adverse growth conditions, its performance may be poor. In growing trials, Maja has appeared to crop rather more heavily than Kenia, to which it is closely related, but its straw seems somewhat weaker. In addition to the information summarized here, useful general information is given as to diagnostic features among barley varieties, and a number of well-produced plates add very considerably to the value of the publication.

YEAST AS AN AID IN THE WORLD'S FOOD SUPPLY PROBLEM

Much has been done by men in many countries on the production and use of yeast for food, and we have been told that the efficiency of the yeast cell as a builder of protein far outstrips that of the bullock. In a world where the supply of food, especially protein, is lagging more and more behind demand, this fact becomes of increasing importance, and *Chemical and Engineering News* (22nd Nov., 1948, p. 3487) reports a symposium of biochemists and dieticians held in Milwaukee last November to discuss the use of yeast in feeding. Its nutritive value is established, and the problem now is how best to make it palatable and readily usable. Recent developments in the processing of brewers' yeast—debitting, drying, smoking and other treatments—have gone a long way to solve the problem.

Brewers' yeast is, of course, available only in comparatively small amount, and to meet an increased demand yeast is now being produced from waste wood-cellulose and canning wastes, with the addition of the necessary minerals and some simple form of nitrogen, as well as from the usual molasses. The cost of making yeast from wood wastes if plant, labour and overhead expenses are excluded, has been put at a little under a penny a pound. There are four million tons of canning wastes available annually in the United States and sugar from these can be supplied at about a farthing a pound to a yeast factory, where it can be converted into yeast with over 50 *per cent.* efficiency.

Not only is yeast a valuable source of protein but also of vitamins, minerals and fats, and it is now possible to grow strains of yeast which will provide important quantities of almost any constituent of the diet. Some strains, for example, can produce as much as 60 *per cent.* of their dry weight as fat, at conversion efficiencies up to 18 *per cent.* of the carbohydrate consumed. Truly a wonderful microcosm.

* * * *

MEASUREMENT OF FOOD ACCEPTANCE

Most people in this country in these days of austere living would, if asked why they accepted a certain food or drink, reply rather

shortly that it was because they had no alternative. We hope and believe, however, that the time will eventually come when personal choice, and not merely availability, will once more be what advertisers are fond of calling a sales factor. In the meantime it is pleasant to give a little thought to what it is that will help determine our choice, and how those whose business it is to meet it can best gain foreknowledge of our reaction.

Appearance and flavour are of paramount importance. The insistence that a particular article is good for you is psychologically excellent, but it would not get very far unless the article appealed also to the eye and the palate. Appearance is objective and can be measured by instruments. Flavour is subjective, and its assessment is a matter for the expert taster; yet not for the expert alone, for final judgment lies with the public. This complex subject is considered in a most interesting and informative way in an article by E. C. Crocker, L. B. Sjöström and G. B. Tallman which appeared in a recent number of *Industrial and Engineering Chemistry* (Dec., 1948, pp. 2254 to 2257). Its practical application to beer has been discussed more than once in this *Journal*, and notably by Dr. Helm (1946, 165) who described how to set about getting statistically significant results from tasting tests.

An interesting point made by the present authors is that experts and trained panels of tasters become relatively objective by reason of their highly selective—one might almost say analytical—palates, and stand in danger of adopting standards of judgment which are not those of the average consumer. This is particularly so when information is being sought of the way in which the consumer will react towards a new article, or change of name, appearance, or flavour. To avoid this danger it is not suggested that the experts should try and work more subjectively, for their very expertness separates them from the average, but that their findings should be supplemented by those of a representative cross-section of potential customers. When this is done, skilled men are needed to design the tasting tests and to interpret the results. The conditions of tasting also need careful adjustment where consumers are involved. We know, for example, how ready the beer drinker is to let type or intensity of colour influence his judgment of strength and flavour. For consumer tests the setting

should be as natural as possible, and the taster should not be asked to judge more than two samples at once. He should not attempt an evaluation or comparison, but be asked to make a clear-cut acceptance or rejection. His reasons for his like or dislike are seldom simple, but they may be important if an effort is being made to modify the consumer's taste, and a skilled questioner will be needed to uncover them.

Perhaps the most reliable way of finding what suits the consumer's taste is to place the article on sale in the normal way in a test area. Unfortunately, this is also accompanied by the greatest financial risk, and whether he succeeds or fails the producer may not know why.

* * * *

ANNUAL REPORT, 1947, OF THE EAST MALLING RESEARCH STATION

During 1947 the area under hops at East Malling remained at 9½ acres, and only three varieties in the seedling garden were grubbed to make room for new selections. The demand for sets of the chief New Varieties still far exceeds the supply. The crop was a good one, although the effects of drought and an infection with red spider were becoming apparent by the end of August. It was noticed in some cutting trials that red spider damage was much more severe on hills cut in December and February from which the most forward bine had not been removed. Detailed records of the seven clonal selections of Goldings have been continued, and 14 Fuggle plants from about 100 selections that are being studied showed promise.

Eight new outbreaks of *Verticillium* wilt were reported from Kent and Sussex during the year, and four milder outbreaks from the West Midlands. The varieties previously showing resistance to this disease maintained their resistance on all the trial sites. Although OB53 and OM26 are the most resistant of these varieties, OR55 and OJ47 appear to be the best from a cultural point of view. Collaborative experiments that were carried out at Long Ashton and Wye on the relation of nutrition with wilt incidence have shown a striking correlation between nitrogen manuring and both incidence and intensity, and from another experiment it

seems that late dressing and training of the vines has the effect of reducing the number of plants showing symptoms of wilt, although it does not affect the severity of the symptoms.

An experiment to find whether nitrogen and potash manuring have any influence on the intensity or spread of nettlehead has also been started in a commercial garden. In a further examination of a number of New Varieties for resistance to graft infection with this disease, none was immune or completely tolerant, but there is evidence that at least one variety, Concord, may show symptoms less readily than the Fuggle. This might lead to some control of nettlehead, but at the same time it could be a danger to other varieties nearby, since infected Concord plants would be more difficult to detect and remove. Aerial surveys and subsequent ground mapping of the distribution of plants infected with nettlehead in a large hop farm in 1946 led to the interesting discovery that the infected plants were especially concentrated on the sites of former hedgerows and pasture fields. Nothing is known, however, of the soil and plant conditions which used to prevail on those sites, and other cases are known in which hops have been planted where hedgerows had been grubbed and have remained free from the disease.

During the year two disorders of unknown cause were noted on hops in many districts. One, "leaf curl," caused the leaves to curl downward and become dark green with necrotic flecks; the other was characterized by the presence of irregular "oak leaf" chlorotic patterns on the leaves. Experiments have shown that split leaf blotch is graft transmissible. Several severe outbreaks of *Phytophthora* root rot were noted, during early September, in the variety Mathon in Worcestershire. This is the first record of this disease causing extensive damage among Mathons. On another farm up to 20 *per cent.* of the plants were affected in plots of certain New Varieties, notably AEE44 and Bullion.

The Report concludes with two articles of interest to hop growers. In the first, measures for the control of hop red spider are brought up to date by A. M. Massee. Dr. Massee recommends the application of a lime sulphur or derris spray in May or early June, when the lower leaves of the vines have been stripped but before they reach the

breast wire. If circumstances demand it a second spray may be given ten days later. Nicotine may be added to either spray when the hop damson aphid or hop fly is present, and in gardens which have suffered severe infestation with red spider the practice of broadcasting naphthalene directly after picking is recommended. In the second article W. G. Keyworth gives a detailed account of three methods of hop propagation by layering, for the guidance of those who wish to raise selected stocks. In layering, which can be done with equipment normally used by hop growers, several feet of bine from each plant are buried, and some 50 cuttings may be obtained at maturity. In this way it is possible to multiply stock very considerably in one season, and a special project was started during 1947 to study improved methods of layering, using the wilt-resistant varieties OR55 and OJ47.

* * * *

THE SECTIONS

MIDLAND COUNTIES SECTION

At a meeting of the Section held on 17th February under the Chairmanship of Mr. B. Wood, Prof. J. de Clerck delivered a lecture entitled "General Developments in Belgian Brewing Technology during and since the War," in which he said that in Belgium, during the present century, top fermentation beer has been steadily supplanted by lager and draught by bottled beer. During the recent war gravities fell to 1009-1019, and beetroot up to 50 *per cent.* was effectively employed when sugar and malt supplies ran low. Post-war conditions have brought high taxation, reduced consumption, and intense competition. In malting, the main development has been the careful restriction of respiration towards the end of the growing period, the Saladin system being still the vogue. Other devices for reducing malting loss are being investigated.

The work of Govaert, which has led to possible economies in hops, is concerned with the artificial transformation of humulon into the more soluble and bitter isohumulon, thus avoiding loss through the formation of non-bitter resins. Pure culture top yeasts have often been found to acclimatize themselves to worts of a given gravity, even a very low one, and the occasional use of a stronger

wort to nourish the yeast was of doubtful benefit. Problems in the conditioning and maturing of beer were discussed, and a description was given of a new patent cask which can be filled without loss of gas or exposure to air.

SCOTTISH SECTION

The Annual General Meeting of the Section was held at the North British Hotel, Edinburgh, on 18th January, 1949, Mr. L. Fletcher presiding. After adoption of the Annual Report and Accounts, the following were elected for the current session:—Chairman: Mr. L. Fletcher; Vice-Chairman: Mr. A. Clark Doull; Committee: Messrs. J. H. Melville, A. Davidson, I. A. Preece, M. R. Dewar, J. B. Laing, J. B. Hamilton Meikle, A. Renton, R. McKinlay, S. J. M. Foote and J. Westwood; Representative on Council: Mr. A. Tait; Representative on Examinations Committee: Mr. A. Clark Doull; Hon. Auditors: Messrs. E. Baxter and J. H. Melville. The Hon. Secretary and Treasurer is Mr. J. Whitton, C.A., 3, St. Colme Street, Edinburgh.

At a subsequent Ordinary Meeting a paper, entitled "Barley," was read by Mr. A. M. Slight, who began by describing the morphological characters of barley and then passed

to practical matters. He said that to select the best and reject the poorer samples offered in the market is a responsibility which calls for all the skill and experience at the maltster's command. In the visual examination of barley samples, the points to be taken into account are: ripeness, condition, moisture content, appearance of skin, regularity in size and shape, purity of race, skinned and damaged corns; details in the technique of assessment were described. The speaker expressed the opinion that the bulk of the Kenia and Maja barleys grown in Scotland were of poor quality, and asked whether the maltster should not now attempt to guide farmers back from high yield to high quality. A vigorous discussion followed.

At a meeting of the Section held in the North British Hotel, Edinburgh, under the Chairmanship of Mr. L. Fletcher, on 15th February, Dr. I. A. Preece delivered a lecture entitled "Diastatic Power and the Mash Tun," describing research work recently carried out in Edinburgh on starch conversion brought about by the joint action of α - and β -amylases (*cf.* this *Journ.*, 1949, 9). The discussion which followed concerned itself chiefly with the possible interdependence of proteolytic and amylolytic changes during malting and mashing.

ERRATUM

Jan.-Feb. issue, 1949, page 42:—line 7 from foot of first column should read: "closed, so that no agitation takes place when".

ANNUAL REPORT OF THE COUNCIL OF THE INSTITUTE OF BREWING

For the Year ended 31st December, 1948

The Council of the Institute of Brewing presents the following Report for the year ended 31st December, 1948:—

ANNUAL GENERAL MEETING

The forty-fifth Annual General Meeting of the Institute of Brewing was held at the Goring Hotel, Grosvenor Gardens, London, S.W.1, on Tuesday, the 25th of May, 1948, the President, Mr. M. V. COURAGE, presiding.

The following were elected at that meeting:—

President: Mr. MAURICE VANDELEUR COURAGE.

Hon. Treasurer: Mr. SANDERS WATNEY, B.A.

Life Members: Messrs. PERCY SMALLEY DOCKRAY, ARTHUR VENN GREENSTREET, HORACE ROWLAND HILL, ERNEST LE MAY (the late), JOHN ROYLE LINNELL, DAVID MARTIN (the late), and ARTHUR MITCHELL.

Ordinary Members of Council: Commander ALAN CORY-WRIGHT, D.S.C., R.N. (retd.), JAMES GRANT, HUGH EDWARD KELLY, F.R.I.C., and RICHARD SELIGMAN, Ph.D., F.C.G.I., F.I.M.

The Meeting was preceded by the customary Annual Meeting of Corporate Members.

SPECIAL GENERAL MEETING

A Special General Meeting was held at the Goring Hotel on the 22nd June, 1948, for the purpose of confirming the Resolutions passed at the Annual General Meeting in regard to (a) the registration of Student Members, and (b) the retirement of members of the Council.

THE COUNCIL

The Council for the year 1948-49 is as follows:—

President: M. V. COURAGE.

Past Presidents: C. H. BABINGTON, CECIL E. W. CHARRINGTON, M.C., G. T. COOK, CHRISTOPHER GEORGE, H. W. HARMAN, Sir SYDNEY O. NEVILLE, Colonel J. H. PORTER, D.S.O., ROBERT V. REID, W. SCOTT, JAS. STENHOUSE, A.C.G.I., and R. J. B. STOREY.

Vice-Presidents: JULIAN L. BAKER, F.C.G.I., F.R.I.C., R. H. BUTLER, Lt.-Col. E. N. BUXTON, M.C., and E. M. STROUTS.

Hon. Treasurer: S. WATNEY, B.A.

Chairman of the Research Fund Committee: Col. W. H. WHITBREAD, T.D., B.A.

Ordinary Members: J. B. BINNIE, Sqd/Ldr. F. H. BOWYER, M.B.E., F. G. BURDASS, Comdr. A. CORY-WRIGHT, D.S.C., R.N. (retd.), H. J. COX, J. G. DANIELL, JAMES GRANT, R. W. HILL FORSTER, H. B. HUTCHINSON, Ph.D., H. E. KELLY, F.R.I.C., Col. B. E. OLIVER, R. SELIGMAN, Ph.D., F.C.G.I., F.I.M., N. B. SMILEY, B.A., W. T. SMITH, and Col. W. H. WHITBREAD, T.D., B.A.

Representatives of the Sections: B. M. BROWN, B.Sc., F.R.I.C., J. A. BURNS, A.R.I.C., and HAROLD HERON (London Section); R. M. FERGUSON, B. J. HERROD, M.C., and R. W. HYDE (North of England Section); E. A. BRAITHWAITE, A. J. C. COSBIE, B.Sc., A.R.I.C., and R. S. SANDBACH (Yorkshire and North-Eastern Section); A. G. P. SMITH, S. H. THOMPSON and B. WOOD (Midland Counties Section); LOUIS FLETCHER, F.R.I.C., A. TAIT and J. WHITTON (Scottish Section); Major L. H. FINCH, M.B.E., T.D., J. H. ST. JOHNSTON, and A. E. TAYLOR, A.R.I.C. (Burton-on-Trent Section).

TRUSTEES

The Council regrets to record the retirement, owing to ill-health, of Mr. R. J. B. STOREY from the office of a Trustee of the Institute of Brewing. Mr. Sanders WATNEY, the Hon. Treasurer of the Institute, has been elected a Trustee in his place and stead. The remaining Trustees are Lt.-Col. E. N. BUXTON, M.C., Mr. Cecil E. W. CHARRINGTON, M.C., and Mr. Arthur MITCHELL.

OBITUARY

The Institute has suffered great loss by the death, during the year, of the following Life Members of the Institute of Brewing:—

ERNEST LE MAY (Member of the Council: 1928–34).

DAVID MARTIN.

WALTER ALFRED RILEY (a Vice-President since 1925).

ROWLAND WHITAKER (a Vice-President since 1932).

SIR OWEN WILLIAM WIGHTMAN, C.B.E.

The Council regrets to record the deaths also of the following subscribing Members:—

BAXTER, JAMES SNEDDON.

BAYLIS, GEORGE EDWARD.

COLLIS, D'ARCY GILBERT.

GOUGH, HENRY.

HOYLE, LESLIE.

JONES, ROBERT THOMAS TAYLOR.

MIEDL, EGON.

OETTINGER, ADOLPH E., Jr.

SIAU, RAYMOND LOUIS.

SIMPSON, ROBERT TELFER.

THOMSON, DAVID S.

NEW MEMBERS

The Council is pleased to record the election of the undermentioned Student, Ordinary, Associate, Diploma and Corporate Members, respectively, of the Institute of Brewing.

Section

Student Members:

Y. & N.E.	ALLMAN, Jack. Student, University of Birmingham.
Lond.	BATEMAN, George Gunson. Pupil with J. W. Green, Ltd., Luton.
N. Eng.	CALDERBANK, Thomas Geoffrey. Student, Bolton Municipal Technical College, Bolton.
Y. & N.E.	CHAMBERLAIN, Walter. Student, University of Birmingham.
Scot.	DYKES, James Edward Townley. Student, Heriot-Watt College, Edinburgh.
Scot.	FALCON, Michael Gascoigne. Pupil, Geo. Younger & Son, Ltd., Alloa, N.B.
Scot.	FERGUSON, Thomas Crawford. Student, Heriot-Watt College, Edinburgh.
N. Eng.	GLEESON, Kenneth. Student, Heriot-Watt College, Edinburgh.
Lond.	HARPER, Norman Williams. Pupil, McMullen & Sons, Ltd., Hertford.
Lond.	HAVERS, David Nicholas Oldfield. Undergraduate, Corpus Christi College, Cambridge.
Scot.	HYDE, Richard John. Student, Heriot-Watt College, Edinburgh.
Mid.	MARTIN, Evan Charles Bonfoy. Pupil, Wrexham Lager Beer Co., Ltd., Wrexham.
Scot.	MCDONALD, Eric Young. Student, Heriot-Watt College, Edinburgh.
Scot.	MEPHIUS, Ronald George Lund. Student, Heriot-Watt College, Edinburgh.
Lond.	RIDD, Allan Charles Roberts. Student, University of Birmingham.
Lond.	STEER-JONES, Peter Steer. Pupil, Style & Winch, Ltd., Maidstone.
Scot.	STEVENSON, Alexander. Student, Heriot-Watt College, Edinburgh.
N. Eng.	TOYER, Peter Barrington. Pupil, Messrs. Heron & Cosbie, Manchester.
Lond.	WAKE, Kenneth Hereward. Pupil, Ind, Coope & Allsopp, Ltd., Romford.

Student Members Elected to Ordinary Membership:

B.-on-T.	BISHOP, Ronald Harry. Asst. Maltster, Ind, Coope & Allsopp, Ltd., Burton-on-Trent.
B.-on-T.	BUNTING, Geoffrey Leonard. Under Brewer, Castle Brewery, Salisbury, Southern Rhodesia.
Y. & N.E.	LANGFORD, Ronald. Asst. Brewer, Workington Brewery Co., Ltd., Workington.

Ordinary Members:

N. Eng.	ANGOOD, William Bernard. Head Brewer, Joseph Holt, Ltd., Manchester.
B.-on-T.	ARMSTRONG, Matthew, C.A. Asst. Accountant, Ind, Coope & Allsopp, Ltd., Burton-on-Trent.
Mid.	ASTBURY, Frank Nicholas, M.Sc. (Columbia), B.Arch. (Liverpool), A.R.I.B.A. Supervising Architect and Joint General Manager, Mitchells & Butlers, Ltd., Birmingham.
Y. & N.E.	BACON, Henry. Asst. Brewer, Workington Brewery Co., Ltd., Workington.
Lond.	BAETSLE, Raf. Director of the "Institut Supérieur des Fermentations," Ghent, and the "Laboratoire de Chimie et Bactériologie de la Ville de Gand," Ghent.

Section

Ordinary Members—continued

- Y. & N.E. BAIN, William Alexander. Head Brewer, Russells & Wrangham, Ltd., Malton.
 B.-on-T. BAMFORD, Oswald Joseph. Brewer, Bass, Ratcliff & Gretton, Ltd., Burton-on-Trent.
 Lond. BARROW, Phillip Owen Hugonin. Brewer, Watney, Combe, Reid & Co., Ltd., London.
 Mid. BEECH, Frederick Walter. Research Chemist & Microbiologist, Goldwell Farms, Ltd., East
 Malling, Kent.
 Lond. BERESFORD, Alan Donald. Asst. Brewer, Ind, Coope & Allsopp, Ltd., Romford.
 Mid. BIRD, Norman Frank. Proprietor, Crown Brewery, Bloxwich, Staffs.
 N. Eng. BOARDMAN, Henry. Manager and Secretary, Volunteer Brewery, Bromley Cross, nr. Bolton.
 Lond. BOTTEN, Arthur William, A.M.I.Mech.E. Managing Director, Bar Equipments, Ltd., London.
 N. Eng. BOTTOMLEY, Claud McClellan, B.Sc. (Lond.), Head Brewer, Ind, Coope & Allsopp, Ltd.,
 Liverpool.
 Lond. BOUGHTON, Alan John. Brewer, Brandon's Putney Brewery, Ltd., Putney.
 Mid. BOUGHTON, Denis. Asst. Brewer, John Joule & Sons, Ltd., Stone.
 Lond. BRADFER-LAWRENCE, Harry Lawrence. Chairman and Managing Director, Hammond's
 United Breweries, Ltd., Bradford.
 N. Eng. BRADLEY, Derek Cecil. Pupil Brewer, Dutton's Blackburn Brewery Co., Ltd., Blackburn.
 B.-on-T. BROOMFIELD, Leslie Walter. Bottling Manager, Ind, Coope & Allsopp, Ltd., Burton-on-Trent.
 Lond. BROWN, John Falcon, D.S.O. Brewer, Arthur Guinness, Son & Co., Ltd., London.
 Lond. BUCKLEY, Eric Sydney. Chemist, Sutton & Phillips, Ltd., Stowmarket, Suffolk.
 Lond. BUTLER, Francis John. Brewer, Ind, Coope & Allsopp, Ltd., Romford.
 Mid. BUTLER, Peter Desmond. Under Brewer, Mitchells & Butlers, Ltd., Birmingham.
 Mid. CARTER, William Albert. Asst. Brewer, Mitchells & Butlers, Ltd., Birmingham.
 Scot. CLUTTERBUCK, Edmund Harry Michael, B.A. (Oxon). Junior Brewer, William Younger & Co.,
 Ltd., Edinburgh.
 Lond. COBB, Francis Mars Den Peter Carr. Pupil, Northampton Brewery Co., Ltd., Northampton.
 Lond. COMPTON, John, B.Sc. (Lond.). Asst. Chemist, Courage & Co., Ltd., London.
 Mid. COOPER, Thomas Leslie Brian, M.A. Asst. Engineer, Mitchells & Butlers, Ltd., Birmingham.
 Lond. COUTANCHE, John Alexander Gore. Pupil, Messrs. Moritz & Fuller, London.
 Lond. COX, Ernest Charles Stanley. Chief Chemist and Works Manager, Fowler, Ltd. (Sugar Manu-
 facturers), London.
 Lond. CRONYN, John Bruce, B.A.Sc. Apprentice Brewmaster, John Labatt, Ltd., London, Ontario.
 Lond. CROSBY, David Douglas. Improver, Meux's Brewery Co., Ltd., London.
 Lond. DALY, Francis Joseph Dooner, A.R.I.B.A. Architect (Private Practice), London.
 Lond. DAWES, Anthony. Asst. Brewer, Wells Watford Brewery, Ltd., Watford.
 Y. & N.E. DAWSON, George. Managing Director, Dawson Bros., Ltd., Gomersal, nr. Leeds; and Hunslet
 Engine Works, Leeds.
 Lond. DAWSON, William Brooke, Asst. Brewer, New Zealand Breweries, Ltd., Wellington, New
 Zealand.
 Y. & N.E. DOTTRIDGE, Fred John. Director, R. & G. Boby Bros. & Chapman, Ltd., Bury St. Edmunds.
 Lond. DUNCAN, Sholto Russell. Brewer, Nelson Breweries, Ltd., Nelson, New Zealand.
 Mid. EDGELL, John Llewellyn. Technical Representative, Liquid Carbonic Co., Ltd., London.
 Mid. EDMONDS, Denis Harcourt, B.A. (Lond.). Student, University of Birmingham.
 N. Eng. EDWARDS, Harry Stephen. Brewer, Bell & Co., Ltd., Stockport.
 Lond. ENGLUND, Gunnar. Managing and Technical Director, Ostersunds Brewery, Inc., Stromsunds
 Brewery, Inc., and Svegs Brewery, Inc., Sweden.
 Mid. ENSOR, John Duncombe. Student, University of Birmingham.
 Y. & N.E. EUSTACE, David Langton. Chief Chemist and Asst. Malting Manager, Wm. Glossop & Bulay,
 Ltd., Hull.
 Mid. FENDICK, Jack Ronald. Surveyor, Mitchells & Butlers, Ltd., Birmingham.
 B.-on-T. FORSHALL, Samuel, M.B.E., B.A. (Cantab.). Solicitor, Burton-on-Trent.
 Y. & N.E. GARTHWAITE, Norman. Head Brewer and Manager, H. B. Clark & Co. (Successors), Ltd.,
 Wakefield.
 Lond. GIBSON, Joseph Henry. Technical Branch, Liquid Carbonic Co., Ltd., London.
 Lond. GLOVER, Harold Henry. Director, Edward Strauss & Co. (Hop Merchants), Ltd., London.
 Scot. GRAHAM, Richard. Junior Brewer, Wm. Younger & Co., Ltd., Edinburgh.
 Mid. GRANT, Edward Kendrick. Under Brewer, Mitchells & Butlers, Ltd., Birmingham.
 N. Eng. GRIME, William Hordley, O.B.E., T.D. District Sales Manager, Cannington, Shaw & Co., Ltd.,
 and the United Glass Bottle Manufacturers, Ltd., Manchester.
 Lond. GRUNDY, Gerald Cecil. Brewer, Watney, Combe, Reid & Co., Ltd., London.
 Lond. HAMMOND, John Edward. Asst. Brewer, J. W. Green, Ltd., Luton.
 N. Eng. HAMPSON, John. Chairman and Managing Director, Hydes' Anvil Brewery, Ltd., Manchester.
 Lond. HARPER, Gordon Eugene. Brewer, Hodgson's Kingston Brewery Co., Ltd., Kingston-on-
 Thames.
 Mid. HARRIS, Richard Stuart McRae. Asst. Engineer, Mitchells & Butlers, Ltd., Birmingham.
 Lond. HERON, John Richard, B.Sc. (Lond.), A.R.C.S. Student, Messrs. Heron & Cosbie, London.
 N. Eng. HODGES, Joseph Ernest. Asst. Head Brewer, Wilson's Brewery, Ltd., Manchester.
 Mid. HODGSON, John Arthur Stanley, M.A. (Cantab.), A.M.I.Mech.E. Engineer, Ansell's Brewery,
 Ltd., Birmingham.

Section

Ordinary Members—continued

- N. Eng. HOPKINS, Andrew Don Wystan. Pupil, Groves & Whitnall, Ltd., Salford.
 Lond. HORNSEY, Harold William. General Manager, Gaskell & Chambers, Ltd., London.
 N. Eng. HUGHES, William Victor. Pupil, Greenall, Whitley & Co., Ltd., Warrington.
 Lond. HUTCHENS, Frank Stanley. Sales Manager, British Filters, Ltd., Maidenhead.
 Scot. KERR, Archibald James. Junior Brewer, Wm. Younger & Co., Ltd., Edinburgh.
 Lond. KNIGHT, Alfred George. Asst. Brewer, Whitbread & Co., Ltd., London.
 Lond. KNIGHT, Edwin Coulthard, Ph.D., M.Sc.Tech. (Manchester), A.R.I.C. Senior Chemist,
 Arthur Guinness, Son & Co., Ltd., Dublin.
 Lond. LAWDHAM, Charles Howard. Manager, Defiant Crown Co., Ltd., London.
 Lond. LAWRENCE, William Charles. Bacteriologist and Chemist, Malayan Breweries, Ltd., Singapore.
 Mid. LEEK, Gordon Harry. Student, University of Birmingham.
 N. Eng. LEES-JONES, John Richard. Brewery Management, J. W. Lees & Co. (Brewers), Ltd., Man-
 chester.
 Lond. LEWIS-JONES, William, A.M.I.Mech.E. Engineer, Bristol Brewery, Georges & Co., Ltd.,
 Bristol.
 Lond. MACKENZIE-CHARRINGTON, Angus Keith Ingleby. Brewer, Charrington & Co., Ltd., London.
 Lond. MAIDEN, Alan Mulock, Ph.D. (Liv.), B.Sc. (Liv.), F.R.I.C. Development Chemist, George
 Clark & Son, Ltd., London.
 Lond. MAIDMENT, Charles Eric, M.A. (Cantab.). Brewer, Fremfins, Ltd., Maidstone.
 Y. & N.E. MAULE, Aidan Bernard. Director, Head Brewer and General Manager, Sheffield Free Brewery
 Co., Ltd., Sheffield.
 Scot. MCINTOSH, Thomas Pearson. Director, Seed Testing Station, Edinburgh.
 Y. & N.E. MEAKINS, John Derek. Asst. Brewer, W. B. Reid & Co., Ltd., Newcastle upon Tyne.
 Lond. MENG, Gerhard. Brewmaster, Schoenling Brewing Co., Cincinnati, U.S.A.
 Lond. MIDGLEY, Percy, A.M.I.Mech.E. Chief Engineer and Director, Liquid Carbonic Co., Ltd.,
 London.
 Lond. MORRIS, Albert Edward. Brewer, Watney, Combe, Reid & Co., Ltd., Mortlake, S.W.
 Mid. MORRIS, Owen, B.Sc. (Birm.), A.R.I.C. Student, University of Birmingham.
 Mid. MUEHL, Flory Michael. Asst. Brewmaster, Miller Brewing Co., Milwaukee, Wisconsin, U.S.A.
 Lond. NEUBAUER, Carl Walter. Chemist and Master Brewer, Burger Brewing Co., Cincinnati, U.S.A.
 Y. & N.E. NICHOLSON, Philip Martin. Asst. Brewer, Sheffield Free Brewery Co., Ltd., Sheffield.
 Lond. NORTHAM, Paul Cavell. Pupil, Courage & Co., Ltd., London.
 Lond. O'BRIEN, Edward Charles, B.Sc. (N.U.I.) Asst. Chemist and Pupil Brewer, Beamish & Craw-
 ford, Ltd., Cork.
 Mid. OEHMICHEN, Herbert K. Asst. Brewmaster, Miller Brewing Co., Milwaukee, Wis., U.S.A.
 Lond. PALMER, Adrian Horsley. Under Manager, T. W. Wilson & Sons, Ltd., Maltsters, Hadleigh,
 Suffolk.
 Mid. PATTERSON, Michael Leo, B.A. (Oxon.), Asst. Brewer, Mitchells & Butlers, Ltd., Birmingham.
 Scot. PHILP, Robert. Works Manager, Caledonian Distillery (Distillers Co., Ltd.), Edinburgh.
 Y. & N.E. PORTEUS, George, jun. Technical Representative, George Porteus & Sons (Leeds), Ltd.,
 Leeds.
 Lond. PRETER, Franz Florent C. de. Brewmaster, Herenta Psebann 415, Deurne Zuid, Antwerp.
 Lond. ROCHE, Andrew Keiser Shriver. Managing Director, P. J. Roche & Sons, Ltd., Maltsters,
 Enniscorthy.
 Lond. ROGERSON, Barry Hugh. Malting Student, J. Harrington & Sons, Ltd. (Maltsters), Hertford.
 Lond. ROWLEY, John. Pupil, Greene, King & Sons, Ltd., Cambridge.
 Lond. SAMUELS, James Valentine. Head Brewer, Castle Brewery, Salisbury, S. Rhodesia.
 Lond. SAUNDERS, Harry Giles Scott. Asst. Brewer, Chas. Hammerton & Co., Ltd., London.
 Lond. SEMLER-JORGENSEN, Arne. Technical Director, C. Wiibroes Brewery, Helsingor, Denmark.
 Scot. SHADAKSHARASWAMY, Mallappa. Research Student, Heriot-Watt College, Edinburgh.
 Lond. SHILDRICK, Lancelot Richard. Brewer, Arthur Guinness, Son & Co., Ltd., London.
 Mid. SILVEY, Bernard Leigh. Works Manager, Bottling Department, J. Davenport & Sons
 Brewery, Ltd., Birmingham.
 Mid. SIMPKISS, Horace Leslie. Chief Clerk, Brewing Administration Office, Mitchells & Butlers,
 Ltd., Birmingham.
 Mid. SMITH, George Victor. Managing Clerk (Buying Department), Mitchells & Butlers, Ltd.,
 Birmingham.
 B.-on-T. SMITH, Keith Basil. Maltster, Bass, Ratcliff & Gretton, Ltd., Burton-on-Trent.
 Scot. SMITH, Robert. Analyst, Thos. Bernard & Co., Ltd., Leith, N.B.
 Y. & N.E. STEWARDSON, Donald McIntosh. Northern Representative for A.P.V. Co., Ltd., Sheffield.
 Mid. SWANSON, John Gordon. Outside Manager, Ansells Brewery, Ltd., Birmingham.
 Lond. SWINSCOW, Alleyne Godfrey. Brewer, Whitbread & Co., Ltd., Hythe.
 Lond. TAYLOR, Leonard, B.Sc. (Lond.), A.R.I.C. Chemist, Courage & Co., Ltd., London.
 B.-on-T. THOMPSON, Neville Guy. Managing Director, Ind, Coope & Allsopp, Ltd., Burton-on-Trent.
 Lond. TOOBY, Hubert John. Hop Grower, Bransford, Worcester.
 Lond. TURNER, Lewis John. Technical Managing Director, Fuller, Smith & Turner, Ltd., Chiswick.
 B.-on-T. WALLACE, David Dempsey. Brewer, Ind, Coope & Allsopp, Ltd., Burton-on-Trent.
 B.-on-T. WATSON, Michael Gerard Arthur. Trainee, Ind, Coope & Allsopp, Ltd., Burton-on-Trent.

Section

Ordinary Members—continued

Mid.	WEBB, Michael John. Director, Edward Webb & Sons (Stourbridge), Ltd., Stourbridge.
Mid.	WHITEHOUSE, Alan George Raine. Chemist, Mitchells & Butlers, Ltd., Birmingham.
Lond.	WILLIAMS, Stanley Juxon. Governing Director, Samuel Wilkerson & Son, Ltd., Barley Merchants, Royston.
Mid.	WOOD, John Clifford. Secretary, Fredk. Smith, Ltd., Birmingham.

Student Member elected to Associate Membership:

Mid.	ROXBROUGH, John Leonard. Asst. Brewer, H. E. Thornley, Ltd., Leamington Spa.
------	--

Ordinary Members elected to Associate Membership:

Lond.	DARLING, Robert Oswald. Under Brewer, Watney, Combe, Reid & Co., Ltd., Mortlake.
Mid.	GUEST, John Holden Elliot, B.A. (Cantab.). Brewer, Bass, Ratcliff & Gretton, Ltd., Burton-on-Trent.
Y. & N.E.	HERBERT, William John Shearer. Head Brewer, Truswell's Brewery Co., Ltd., Sheffield.
Lond.	HILL, Arnold Maurice. Brewer, J. W. Green, Ltd., Luton.
Y. & N.E.	HOBSON, Derek, B.Sc.Tech. (Manchester), A.M.C.T. Asst. Brewer, John Smith's Tadcaster Brewery Co., Ltd., Tadcaster.
Lond.	JESSUP, George Albert. Brewer, Style & Winch, Ltd., Maidstone.
Y. & N.E.	JOLLEY, Peter William Raby. Brewer, Hewitt Bros., Ltd., Grimsby.
Lond.	MILLER, Noel William. Asst. Brewer, Charrington & Co., Ltd., London.
Lond.	MONCKTON, Herbert Anthony. Brewer, J. W. Green, Ltd., Luton.
B.-on-T.	PENROSE, John Denis Fitz-Gerald. Asst. Brewer, Truman, Hanbury, Buxton & Co., Ltd., Burton-on-Trent.
Lond.	ROBERTS, Richard Henry, B.Sc. (Birm.), Ph.D. (Birm.), Under Brewer, Mitchells & Butlers, Ltd., Birmingham.
Lond.	URQUHART, William Blackie Morrison. Chemist, Truman, Hanbury, Buxton & Co., Ltd., London.

Associate Members:

N. Eng.	BEARDMORE, Donald Joseph. Asst. Brewer, Wilson's Brewery, Ltd., Manchester.
Lond.	GOVERS, Hugh, B.Sc.Agric. (Sydney). Chief Chemist and Asst. Brewer, Toohey's, Ltd., Sydney, Australia.
Scot.	IRELAND, David. Asst. Brewer, Thos. Usher & Son, Ltd., Edinburgh.
Scot.	MACDONALD, Ian Cameron. Pupil, Mackay & Co., Ltd., Alloa, N.B.
Scot.	MCCANDLESS, Robert John. Junior Brewer, Arch. Arrol & Son, Ltd., Alloa, N.B.
Lond.	MCLEOD, Charles Henry. Asst. Brewer, Page & Overton, Ltd., Croydon.
B.-on-T.	MORGAN, Eric John. Asst. Brewer, Ind. Coope & Allsopp, Ltd., Burton-on-Trent.
Lond.	NAUDI, Joseph Hector. Asst. Brewer, Simonds-Farsons-Cisk, Ltd., Malta.
Scot.	RAMSAY, Thomas Gordon. Asst. Brewer, Robert Younger, Ltd., Edinburgh.
Scot.	SUTHERLAND, Hugo, B.E. (Liv.). Asst. Brewer, J. & R. Tennant, Ltd., Glasgow.
Lond.	TADMAN, Cedric Robert. Under Brewer, Northampton Brewery Co., Ltd., Northampton.

Associate Members elected to Diploma Membership:

Mid.	DICKENS, Robert. Improver, Ansells Brewery, Ltd., Birmingham.
Scot.	DOIG, William McLellan. Brewer, Wm. Younger & Co., Ltd., Edinburgh.
Lond.	HILL, Arnold Maurice. Brewer, J. W. Green, Ltd., Luton.
Lond.	MCELWEE, Philip. Brewer, Ann Street Brewery Co., Ltd., Jersey.

Corporate Members:

Mid.	THOMAS & EVANS, Ltd., Barbourne Brewery, Worcester.
Lond.	RUDEBECK, H. & Co., Ltd., Barley Merchants, London.

ROLL OF MEMBERS

The number of subscribing members of the Institute on the 1st January, 1948, was 1,708. Since then 151 new members have been elected. The Institute has, however, lost 11 members by death, and 29 members by resignation and other causes, whilst 7 have been transferred to Life Membership, and 22 desire to commence their membership on the 1st January, 1949.

The number of subscribing members on the books of the Institute on the 31st December, 1948, was, therefore, 1,790, showing a net increase of 120 as compared with 31st December, 1947.

<i>Class of Membership.</i>	<i>31st Dec., 1947.</i>	<i>31st Dec., 1948.</i>
Corporate ..	407	407
Diploma ..	248	234
Associate ..	95	117
Ordinary ..	906	1,003
Student ..	14	29
	<u>1,670</u>	<u>1,790</u>

The number of subscribing members in each Section on the 31st December, 1948, was as follows, viz.:—

<i>Membership.</i>	<i>London Section.</i>		<i>North of England Section.</i>		<i>Yorkshire and North-Eastern Section.</i>	
	<i>1947</i>	<i>1948</i>	<i>1947</i>	<i>1948</i>	<i>1947</i>	<i>1948</i>
Corporate ..	183	182	70	70	56	56
Diploma ..	112	110	31	27	22	20
Associate ..	40	48	12	13	7	11
Ordinary ..	447	500	71	87	79	88
Student ..	6	11	2	4	0	2
TOTALS ..	788	851	186	201	164	177

<i>Membership.</i>	<i>Midland Counties Section.</i>		<i>Scottish Section.</i>		<i>Burton-on-Trent Section.</i>	
	<i>1947</i>	<i>1948</i>	<i>1947</i>	<i>1948</i>	<i>1947</i>	<i>1948</i>
Corporate ..	54	55	35	35	9	9
Diploma ..	29	25	37	34	17	18
Associate ..	9	10	25	30	2	5
Ordinary ..	154	165	91	91	64	72
Student ..	2	3	2	9	2	0
TOTALS ..	248	258	190	199	94	104

HORACE BROWN MEDAL

In the regrettable absence of Mr. Julian L. BAKER through illness, the "Horace Brown Medal," the award of which to Mr. BAKER was mentioned in the last Annual Report, was handed by the President to his son, Mr. Patrick BAKER, at a Special General Meeting of the Institute of Brewing, held at the Royal Institution, Albemarle Street, London, on Monday, 19th January, 1948.

After the presentation, the "Horace Brown Memorial Lecture," which was to have been delivered by Mr. BAKER, was read by Dr. L. R. BISHOP. This lecture, entitled "British Brewing in Retrospect and Prospect," was subsequently published (this *Journal*, 1948, 88).

BOARD OF EXAMINERS

The Board of Examiners for 1948 was as follows:—

Representatives of the Examinations Committee.

R. G. AULT, B.Sc., Ph.D., F.R.I.C.
L. R. BISHOP, M.A., D.Sc., F.R.I.C.

A. CLARK DOULL.
G. T. PEARD, M.Sc., F.R.I.C.

*The Examiners.**First Associateship Examination*

Inorganic and Physical Chemistry	..	Prof. W. WARDLAW, D.Sc., F.R.I.C.
Organic Chemistry		I. A. PREECE, M.Sc., Ph.D., F.R.I.C.
Physics		T. L. IBBS, Ph.D.

Second Associateship Examination

Plant Biology		W. T. WILLIAMS, B.Sc., A.R.C.S., Ph.D.
Organic Chemistry		I. A. PREECE, M.Sc., Ph.D., F.R.I.C.
Plant Biochemistry		L. R. BISHOP, M.A., Ph.D., D.Sc.

Diploma Membership Examination

Malting		Comdr. A. CORY-WRIGHT, D.S.C., R.N. (<i>retd.</i>).
Brewing		B. DIXON.
Brewery and Public House Cellar Management and Cooperage	..	B. DIXON.
Bottling		R. W. HARRIS.
Engineering		W. S. BLACK.
Scientific Control		C. J. VIRDEN, M.A., B.Sc., D.Phil.

June (1948) Examinations.

Examinations were held from the 21st to the 23rd June, 1948. These were the first examinations to be held under the revised Regulations and Syllabus, the major revision being the addition of a second paper in "Organic Chemistry" to the old Part I (b), now the "Second Associateship Examination."

Candidates who had passed Part I (a), now the "First Associateship Examination," at a previous examination, *i.e.*, under the old regulations, were exempted from sitting for the second paper in "Organic Chemistry."

Sixty-four candidates presented themselves in the United Kingdom and one in the U.S.A., that is to say, six in Birmingham (the University), thirty-one in Edinburgh (Heriot-Watt College), sixteen in London (Royal Institute of Chemistry), eleven in Manchester (College of Technology), and one in Chicago (Siebel Institute of Technology).

Of these, eighteen sat for one or more of the subjects of the old Part I by special dispensation; thirty-eight sat for the First Associateship Examination; four, by leave of the Examinations Committee for the First and Second Associateship Examinations; and five for the Diploma Membership Examination.

The following were successful:—

PART I (a)

(3 entered: 3 passed)

- † BROAD, John Russell, Dorchester.
 FULLERTON, Lionel M., Edinburgh.
 SPENCER, Geoffrey Brian, Northampton.
 † *Passed Part I (b) at a previous Examination.*

PART I (b)

(10 entered: 10 passed)

- EWING, Alexander Ferguson Menelaws, Edinburgh.
 HAY, John William Alexander, Edinburgh.
 HOPKINS, Andrew Don Wistan, Manchester.
 LEACHMAN, Jack Ramsay, Northampton.
 LUMSDEN, James Iain Blain, Edinburgh.
 * MACDONALD, Ian Cameron, Edinburgh.
 MCGREGOR, Duncan Grant, Edinburgh.
 McLEOD, Charles Henry, Croydon.
 * RAMSAY, Thos. Gordon, Edinburgh.
 * THOMSON, Robert Morrison, Edinburgh.

PART I (a) and (b)

(5 entered: 2 passed)

- BEARDMORE, Donald Joseph, Manchester.
 NELMES, Derek Arthur, Uxbridge.

FIRST ASSOCIATESHIP EXAMINATION

(42 entered: 23 passed)

- BARBOUR, Thomas Downie, Edinburgh.
 BOARDMAN, William, Bolton.
 * BROWN, James Edward, Sheffield.
 BURTON, John, Newcastle upon Tyne.
 CLARK, John, Birmingham.
 FERGUSON, Thomas Crawford, Edinburgh.
 * HENSON, John Carter, Liverpool.
 I'ANSON, John Alistair Peter, London.
 INSILL, George William Henderson, Edinburgh.
 IRELAND, David, Edinburgh.
 JONES, Claude Barrington, Northampton.
 McCANDLESS, Robert John, Alloa, N.B.
 McDONALD, Eric Young, Edinburgh.
 MALTMAN, James Kelso, Pontefract.
 NEWMAN, Ernest Henry, London.
 NIVEN, Maurice, Sunderland.
 ROE, Gerald Bertram, Faversham.
 * STEPHINSON, Peter Dixon, Sheffield.
 WHITTY, Brian Waltingford, Nottingham.
 WILSON, Peter Louis, Chesterfield.
 WILSON, William, Edinburgh.
 WOODLAND, Raymond, Bridgwater.
 WURZBURGER, Martin.

* "With Distinction."

SECOND ASSOCIATESHIP EXAMINATION

(4 entered: 4 passed)

BAILEY, James Hill, Chiswick.
 BURTON, John, Newcastle upon Tyne.
 IRELAND, David, Edinburgh.
 McCANDLESS, Robert John, Alloa, N.B.

DIPLOMA MEMBERSHIP EXAMINATION

(5 entered: 5 passed)

DEWAR, Maurice Ronald, Edinburgh.
 *DICKENS, Robert, Birmingham.
 *FOOTE, Sydney James Miller, Edinburgh.
 *HILL, Arnold Maurice, Luton.
 *LAING, Hector, Malton.

* "With Honours."

EXAMINATIONS COMMITTEE

Committee: R. G. Ault, B.Sc., Ph.D., F.R.I.C., J. B. Binnie, L. R. Bishop, M.A., D.Sc., F.R.I.C., H. J. Cox (*Nominated by the Midland Counties Section*), J. G. Daniell, Jas. Grant, H. W. Harman (*Chairman*), A. Clark Doull (*Nominated by the Scottish Section*), R. W. Hill Forster, A. L. Jolliffe, A. H. Morris (*Nominated by the North of England Section*), G. T. Peard, M.Sc., F.R.I.C. (*Nominated by the Burton-on-Trent Section*), I. A. Preece, M.Sc., Ph.D., F.R.I.C., J. C. Radcliffe, B.A. (*Nominated by the Yorkshire and North-Eastern Section*), B. V. S. Seed, W. T. Smith, C. J. Virden, M.A., B.Sc., D.Phil., W. J. Watkins (*Nominated by the London Section*), and G. Younie. *Honorary Member:* The President of the Incorporated Brewers' Guild.

Ministry of Labour: Careers Pamphlet No. 8, "Brewing."

On the recommendation of the Examinations Committee the Ministry of Labour has been asked to substitute the following paragraph for those hitherto appearing under "Opportunities for Employment," viz.:—

"Owing to the limited number of vacancies in the Brewing Industry, candidates are advised to get into touch with the Secretary of the Institute of Brewing before embarking on a course of training."

Award under the John S. Ford Memorial Trust.

A Supplemental Deed, necessitated by the substitution of the "First Associateship Examination" for Part I (a) of the prescribed examination, and the substitution of the "Second Associateship Examination" for the old Part I (b) has been duly executed by the Trustees and by the President of the Institute on behalf of the Council.

Clause 3 of the Principal Deed has thereby been varied as follows, that is to say, the words "having previously passed the First Associateship Examination of the Institute have passed the Second Associateship Examination of the Institute in the year in which the award is made" have been substituted for the words "have passed Part I (a) and (b) of the Examinations of the Institute in the year in which the award is made."

By the kind collaboration of everyone concerned, Mr. K. H. SKINNER, to whom the first award under the John S. Ford Memorial Trust was made, was enabled to visit the following Breweries, Laboratories and Maltings in Scandinavia, viz.:—Messrs. Wiibroes Bryggeri, Helsingor; Carlsberg Breweries and Laboratories, Copenhagen; the Jorgensen Laboratory, Copenhagen; the Falken Brewery, Falkenberg; the Carnegie Maltings, Gothenburg; and Aktiebolaget Stockholms Bryggerierne, Stockholm.

An account of this series of visits was given by Mr. SKINNER before the Scottish Section on 19th October, 1948.

A further award under the Trust, that is to say, on the results of the 1948 examinations, has been made to Mr. Robert Morison THOMSON, of Edinburgh.

Arrangements have been made for him to spend some six weeks in the Carlsberg Breweries, Maltings and Laboratories, a similar period in the Stockholm Breweries, and lesser periods in Messrs. Wiibroes Brewery, Helsingor, and in the Carnegie Maltings, Gothenburg.

Reference was made in the last Annual Report to the question whether reciprocal arrangements could not be made for continental students of Brewing in respect of the breweries of Great Britain, and this matter was referred to a Hospitality Committee. (See page 75).

POST-WAR EDUCATION COMMITTEE

Committee: The President and Hon. Treasurer (*ex-officio*), J. B. Binnie, Cecil E. W. Charrington, M.C., Prof. R. H. Hopkins, D.Sc., F.R.I.C., J. M. Inches, I. A. Preece, M.Sc., Ph.D., F.R.I.C., N. B. Smiley, F. A. Smith, W. T. Smith and W. J. Watkins.

Registration of Students.

The Rules and Bye-Laws were altered at the last Annual General Meeting to give effect to the proposals, outlined in the last Annual Report, for the election and registration of Student Members.

Candidates for Student Membership are now required to produce evidence satisfactory to the Council:—

- (a) That they are not less than 16 years of age.
- (b) That they have passed the examination of the School Leaving Certificate, or a recognized equivalent. The Council may, however, in its discretion, accept other evidence of general education.
- (c) That they are recommended for registration as Student Members:—
 - (i) By a teacher of chemistry in any university or other institution in which they are studying for the Institute's Examinations; or
 - (ii) By a Diploma or other Member under whom they are working.

Note.—The Council will require from the Diploma or other Member an assurance that the student will have facilities for obtaining satisfactory experience in general laboratory work in all the subjects of the First and Second Associate-ship Examinations.
- (d) That they are undergoing a course of preparation and training, approved by the Council, with the object of qualifying for the Associate Membership of the Institute of Brewing.

Registered Students are required to comply only with those regulations, governing admission to the Associate Membership Examinations, as are in force at the time of their registration.

Regional Advisory Councils for the Organisation of Further Education.

Regional Advisory Councils have been appointed to ensure, *inter alia*, that the educational needs of the various industries are being met in the colleges and educational institutions within their particular areas and advisory panels have been appointed for each industry.

YORKSHIRE

Towards the close of 1947, the attention of the Council was directed by the "Yorkshire Council for Further Education" to a series of discussions, between its Advisory Committee for Chemistry and the various trades and industries in the Northern part of the West Riding, with a view to ascertaining whether the needs of industry were being met in the technical colleges and institutes of Yorkshire.

At the suggestion of the Institute's Post-War Education Committee, the Yorkshire and North-Eastern Section appointed two representatives to attend a meeting of the aforesaid Advisory Committee held in November, 1947, and Mr. R. W. Hill FORSTER and Mr. R. S. SANDBACH attended the meeting.

These representatives drew attention to the present educational facilities in Birmingham, Edinburgh, London and Manchester, respectively, and suggested that it would be a great advantage to those engaged on the technical side of the Brewing Industry in the North-Eastern Counties if a university or technical college in Yorkshire were to establish courses covering the syllabus of the Institute's examinations.

It was ascertained that the Leeds Technical College had facilities for the training of intending brewers up to Associate Membership standard, but that it would be necessary for the College to have some idea of the number of Candidates who would be likely to attend classes, if instituted, for the Associate Membership Examinations. It was suggested that, if sufficient Candidates were forthcoming, they would be linked up with the classes for medical students which included subjects covered by the Institute's Examinations.

EAST MIDLANDS

At the invitation of the Council, the Burton-on-Trent Section has nominated Mr. F. B. BUXTON, M.Sc., to serve on the Advisory Panel for the Chemical Industry of the Regional Advisory Council for the Organization of Further Education in the East Midlands.

PUBLICATIONS COMMITTEE

Committee: The President and Hon. Treasurer and the Chairmen of Sections (*ex-officio*), L. R. Bishop, M.A., D.Sc., F.R.I.C., B. M. Brown, B.Sc., F.R.I.C., A. J. C. Cosbie, B.Sc., A.R.C.Sc.I., A.R.I.C., Bernard Dixon, A. Clark Doull, H. W. Harman, Harold Heron, Prof. R. H. Hopkins, D.Sc., F.R.I.C., H. E. Kelly, F.R.I.C., J. E. Martineau, R. Seligman, Ph.D., A.C.G.I., F.I.M., and W. T. Smith.

Since 1942, the *Journal* has been published in alternate months. The six numbers published during 1948 contained 344 pages, of which 41 were devoted to "Monthly Review" notes, 173 to papers and communications, 50 to reports published under the auspices of the Research Fund Scheme, and 80 to abstracts of papers published in contemporary journals, and abstracts of patents and reviews of books.

The Council is indebted to the authors of the under-mentioned papers, reports and communications which have appeared in the *Journal* during the year, *viz.*:—

- BAKER, Julian L.: "British Brewing in Retrospect and Prospect." (Horace Brown Memorial Lecture.)
 BISHOP, L. R.: "The Adjustment of Prediction to give True Extract in Malt."
 BISHOP, L. R., CUFF, C. M., and HICKSON, W.: "A Theoretical Basis for the Calculation of True Extract of Malt and its Relationship to Apparent Extract in the Heron Method."
 BISHOP, L. R., and HICKSON, W.: "The Revised Calculation of the Extracts of Malts and Adjuncts in the Institute of Brewing Standard Methods."
 BREMNER, T. S., and KELLY, H. E.: "The Economical Use of Bottle-Washing Detergents."
 BROWN, B. M.: "Research and the Brewer."
 BROWN, B. M., LASMAN, W. C., WILMOT, J. W., and HODGSON, N.: "Brewing Trials with Varieties of Hops showing some Resistance to *Verticillium* Wilt."
 CHERRY-DOWNES, H. A. D.: "Mechanization of Maltings."
 GASCOYNE, G.: "The Season's Hops, 1947."
 HOPKINS, R. H., WIENER, Stella, and RAINBOW, C.: "Pantothenic Acid in Brewing."
 HUNTER, Herbert: "The Influence of Environment on the Yield and Quality of Certain Varieties of Malting Barley."
 JOHNSTON, J. H. St.: "The Separation of the Protein Constituents of Wort."
 KELLY, H. E., and BREMNER, T. S.: "Observations on Malt Analysis Technique."
 KULKA, Dora, TOSIC, J., and WALKER, T. K.: "*Acetobacter* Infection" (*Inst. Brew. Research*); and "A Strain of *Lactobacillus plantarum* suitable for use in the Estimation of Antiseptic Power" (*Inst. Brew. Research*).
 KULKA, Dora, and WALKER, T. K.: "Capsules of *Acetobacter turbidans* 'Demonstrated' by Positive Staining."
 LYNES, K. J., and NORRIS, F. W.: "Biotin in the Materials and Process of Brewing."
 PAINE, S. W. T.: "Observations on the Behaviour of Yeast in Fermenting Worts."
 PREECE, I. A.: "Some Aspects of Stability of Brewery Malt α -Amylase."
 PRICE, M. D.: "The Khapra Beetle."
 SALMON, Prof. E. S.: "Thirtieth Report on the Trial of New Varieties of Hops, 1946."
 SANDEGREN, Evald: "Barley Phytin in Malting and Brewing."
 SELIGMAN, R.: "The Research Scheme of the Institute—its Past and its Future."
 SHIMWELL, J. L.: "A Rational Nomenclature for the Brewery Lactic Acid Bacteria" (*Inst. Brew. Research*); and "A Study of Ropiness in Beer" (*Inst. Brew. Research*).
 THOMPSON, H. L.: "The 1948 Barley Crop."
 WATKINS, W. J.: "Pre-Bottling Pasteurization of Beers and Sterile Filling."
 WHITLEY, W. A.: "Routine Biological Tests in the Brewery."

FUEL ECONOMY COMMITTEE

Committee: The President, the Hon. Treasurer, the Chairman of the Research Fund Committee (*ex-officio*), Major G. S. M. Ashby, Sir Hugh Beaver (*Chairman*), G. T. Cook, A. F. Garton, C. E. Lanfear, J. A. Lones, E. G. Morris, F. E. B. Moritz, Sir Sydney O. Neville, M. W. Plumptre, Walter Scott, and W. T. Smith.

As indicated in the last Annual Report the Committee felt that it would serve no useful purpose, at the then juncture, to re-issue, either in its original or a revised form, the detailed technical information given in its pamphlet on "Fuel Economy," which was first issued in 1918 and reprinted in 1942. A brief memorandum, urging the adoption of certain measures to secure economy in fuel, was, however, prepared and issued to all breweries in March, 1948. When it becomes once more possible to purchase fuel on a definite specification and when the provision of new plant and equipment becomes easier, the Committee will propose to issue a new memorandum on fuel use in the Brewing Industry for long-term application. Meanwhile, the Committee would once more stress how much can be achieved by close study and control, and how necessary and profitable that is.

After consultation with the Brewers' Society it has been agreed that the Institute should deal with matters of research, and that the Society should deal with those aspects of fuel economy that are not primarily matters of research, for the sake of example, the question of the practical application of the results of research, and the questions of propaganda and policy.

It is felt that, apart from research on fuel, *qua* fuel, which obviously falls within the scope of the Ministry of Fuel and Power, there is a number of possible fields for research in connection with the utilisation of steam, heat and energy in breweries, and the hope has been expressed that, in the event of provision being made by the Research Fund Committee for an Engineering Panel, these matters would be adequately covered in a programme of research.

ANALYSIS COMMITTEE

Committee: The President and Hon. Treasurer (*ex-officio*), R. G. Ault, B.Sc., Ph.D., F.R.I.C., L. R. Bishop, M.A., D.Sc., F.R.I.C., B. M. Brown, B.Sc., F.R.I.C., J. A. Burns, A.R.I.C., A. E. Case, B.Sc., F.R.I.C., G. D. Clarkson, A. A. D. Comrie, B.Sc., A.R.I.C., A. J. C. Cosbie, B.Sc., A.R.C.Sc.I., A.R.I.C., H. E. Dryden, H. W. Harman (*Chairman*), Harold Heron, Prof. R. H. Hopkins, D.Sc., F.R.I.C., E. J. Jeffrey, B.Sc., F.R.I.C., H. E. Kelly, F.R.I.C., L. H. Lampitt, D.Sc., F.R.I.C., H. R. Lyell, A.K.C., F.R.I.C., C. W. McHugo, F.R.I.C., J. H. Oliver, B.Sc., Ph.D., D.I.C., F.R.I.C., I. A. Preece, M.Sc., Ph.D., F.R.I.C., A. Slator, D.Sc., F.R.I.C., Adam Tait, F.R.I.C., C. J. Virden, M.A., B.Sc., D.Phil., H. F. P. Webber, B.Sc., A.R.I.C., and E. R. B. Woodward, B.Sc.

Standard Methods of Analysis.

Owing to the gradual depletion of the Institute's stock of reprints of the "Standard Methods of Malt Analysis," which were last published in 1933, an opportunity was taken in April, 1947, to call the Committee together to consider the question as to how far the "Standard Methods" should be revised.

The revised report, which was adopted by the Council on the 27th July, 1948, was published in August (this *Journal*, 1948, 179), the emendations being shown in heavy type.

The Committee is especially indebted to Dr. BISHOP for determining the modification to be made in respect of the volume of grains in the determination of extracts. A paper on this subject by Dr. BISHOP and Mr. W. HICKSON was published in August (this *Journal*, 1948, 189).

The Committee is also indebted to Dr. BISHOP for the extract tables appearing in the Report.

HOSPITALITY COMMITTEE

Committee: The President and Hon. Treasurer (*ex-officio*), Mr. B. M. Brown, B.Sc., F.R.I.C., Mr. A. Clark Doull, Mr. R. W. Hill Forster, and Mr. N. B. Smiley, B.A.

The Committee has considered the questions referred to it under two heads, *viz.*:—
(1) the question of international collaboration in the matter of the exchange of brewing students; and (2) the question of hospitality to distinguished visitors.

The Committee was of the opinion that there should be a joint Committee of the Brewers' Society and of the Institute. The Society has, however, expressed the view that the matter might well be left in the hands of the Institute, although the Society would be glad to co-operate in any useful way.

The following paragraph has appeared in the Annual Report of the Society for 1947-48, *viz.*:—"The Committee (Parliamentary) endorsed a recommendation from the Institute of Brewing as to affording hospitality to overseas brewing visitors, and members of the Society are asked to co-operate as occasion arises."

Exchange of Brewing Students.

A circular letter was issued to all breweries on the 30th September, 1948, to ascertain how many would be willing to co-operate in the scheme and answers in the affirmative have so far been received from some twenty firms.

Arrangements have already been made for the "adoption" in turn of two continental students by several of these firms.

Distinguished Foreign Visitors.

It has been decided that, whilst individual breweries would wish to entertain those distinguished foreign visitors who expressed a desire to visit them, the Hospitality Committee should be responsible for their entertainment whilst in London. In this connection, it has been suggested that it might be of advantage if special arrangements were made by the Committee for the entertainment of distinguished foreign visitors during the week of the Brewers' Exhibition.

INTERNATIONAL EXHIBITION OF PURE AND APPLIED CHEMISTRY

Arrangements were made by the Society of Chemical Industry for a Stand for the exhibition of the publications of various scientific organizations, including those of the Institute of Brewing, at the International Exhibition of Pure and Applied Chemistry held in Charleroi, Belgium, from the 4th to the 20th September, 1948.

Arrangements were also made for a representative of the bodies participating to be present, during the whole of the Exhibition, to deal with enquiries which, it is understood, were numerous.

VIIITH INTERNATIONAL CONGRESS (TECHNICAL AND CHEMICAL) OF THE AGRICULTURAL INDUSTRIES

A Paper on the "Decomposition of Malt Constituents in Mashing with special reference to Amylase Action" was read by Professor R. H. HOPKINS, before the Brewing Section of the VIIth International Congress of Agricultural Industries (organized by the French Ministry of Agriculture), which was held in Paris from the 12th-18th July, 1948.

BRITISH STANDARDS INSTITUTION

Mr. A. J. C. COSBIE and Mr. M. W. PLUMPTON, respectively, have been nominated to represent the Institute on the undermentioned Committees of the British Standards Institution, *viz.*:—

"The Nomenclature and Definitions for Solid Fuel Burning Appliances": Mr. M. W. PLUMPTON.
"Standardization and Co-ordination of Tests of Rubber": Mr. A. J. C. COSBIE.

FEDERATION OF BRITISH INDUSTRIES

Early in 1947, the Brewers' Society referred to the Institute an invitation from the Federation of British Industries to nominate a representative to serve on an "Education Committee," the terms of reference of which were "to consider all questions affecting the education of the staff for industry, other than manual workers." Mr. J. B. BINNIE, who was appointed to represent the Institute on this Committee, retired from the Committee early in 1948, and the Council has appointed Mr. James GRANT in his place and stead.

EUROPEAN BREWERY CONVENTION

Reference was made in the last Annual Report to certain preliminary arrangements for the formation of a European Brewery Convention for the purpose of fostering international contact in the technical fields of malting and brewing, and to the appointment of a Special Committee to consider:—(a) whether the association representing Great Britain should be the Brewers' Society or the Institute of Brewing; (b) the question of an annual subscription; and (c) ways and means for financing the proposed biennial congresses.

With regard to the subscription, the Council of the Convention suggested that the subscriptions should be based on the approximate annual beer production of each country, that is to say, the expenses would be divided as follows:—

Small producers: Norway and Switzerland together	15 per cent.
Intermediate producers: Denmark, Netherlands and Sweden ..	39 ..
Large producers: Great Britain and France	46 ..

The expenses to the end of 1948 have been estimated at 13,000 Dutch guilders or about £1,300.

The recommendations of the aforesaid Special Committee, which have been approved by the Council of the Institute and by the Finance Committee of the Brewers' Society, were (a) that the national association representing Great Britain should be the Institute of Brewing, and (b) that the subscription for the period ending 31st December, 1948, should be £300.

The Institute's representatives on the Council of the Convention are Mr. B. M. BROWN and Mr. N. B. SMILEY, who have attended meetings thereof held in Copenhagen and Paris, respectively, on the 22nd April and 15th November, 1948.

The next Congress will be held in Lucerne from the 29th May to 5th June, 1949. Particulars will be issued as soon as possible.

With regard to the question of financing this and subsequent Congresses, the subscriptions of participants should cover the cost, but, in view of the uncertainty of the size of the attendances, it is suggested that a small guarantee fund should be established in addition. This would be raised from the national associations in the same proportions as the subscriptions to the Convention.

Congresses will be open generally to any persons interested on payment of the subscription, although priority will be given to nominees of members of the Convention.

Two major subjects are to be discussed at the next Congress, *viz.*:—(a) Proteins in Brewing, and (b) Production of sterile beer by means other than pasteurization.

A Committee has been appointed to report on the organization of barley breeding and research in different countries.

A further Committee, to which Great Britain was invited to nominate a representative, has been appointed to consider the question of the standardization of analytical methods. Dr. L. R. Bishop, who was appointed to represent Great Britain, has been elected Chairman of this Committee.

The Sections

LONDON SECTION

Committee: B. M. Brown, B.Sc., F.R.I.C. (*Chairman*), Comdr. A. Cory-Wright, D.S.C., R.N. (*retd. Vice-Chairman*), J. A. Burns, A.R.I.C. (*Hon. Secretary and Treasurer*), C. L. Downing, J. V. Godfrey, H. Heron, M. S. Heycock, M.A., C. E. Lanfear, A. E. Nicholls, J. H. Oliver, B.Sc., Ph.D., D.I.C., F.R.I.C., J. H. Parker, M. W. Plumptre, M.I.Mech.E., J. Tatton Ridd, W. T. Smith, Sanders Watney, B.A., W. A. Whitley, H. D. L. Williams, and T. Wright.

Mr. HERON represented the Section on the Council of the Institute, and Mr. WATKINS represented the Section on the Examinations Committee.

Nine meetings were held during the year, when the following papers were read and discussed:—

BISHOP, L. R.: "Wort Analysis" (24/3/48).

FRENCH, E. W.: "Modern Bottling Plant" (12/4/48).

HUTCHISON, A. O.: "The 1948 Barley Crop in Scotland" (8/11/48).

PAINE, S. W. T.: "Observations on the Behaviour of Yeast in Fermenting Worts" (12/1/48).

PREECE, I. A.: "Diastatic Power and the Mash Tun" (13/12/48).

SCOTT, J. W.: "From Cask to Consumer" (8/3/48).

SHIMWELL, J. L.: "Sources of Bacterial Infection in the Brewery" (11/10/48).

WILD, J. L.: "Brewing Sugars" (9/2/48).

WILKINSON, P. G.: "Water Analysis, with Special Reference to the Control of Brewing Liquors" (25/10/48).

The meetings on 8th March and 11th October were held, jointly, with the London and South of England Section of the Incorporated Brewers' Guild.

NORTH OF ENGLAND SECTION

Committee: R. M. J. C. Ferguson (*Chairman*), T. K. Walker, Ph.D., D.Sc., F.R.I.C. (*Vice-Chairman*), R. W. Hyde (*Hon. Secretary*), A. A. D. Comrie, B.Sc., F.R.I.C., F. J. Chiverton, A. A. Clapham, W. J. Crook, B. J. Herrod, M.C., R. A. Houghton, C. A. Hyde, A.F.C., H. Manley, A. H. Morris, and J. B. Wall.

Mr. B. J. Herrod continued to represent the Section on the Council of the Institute and Mr. A. H. Morris was again nominated to serve on the Examinations Committee.

During the year, the following meetings have been held in conjunction with the Incorporated Brewers' Guild:—

CHERRY-DOWNES, H. A. D.: "1948 Crop Barleys" (28/10/48).

CLARKE, S. T.: "Pressure Fermentation and Yeast Characteristics" and "Film of the Floating Brewery" (5/2/48).

COSBIE, A. J. C.: "Comments on American Brewing Methods" (2/12/48).

MILLS, W. A.: "Thermal Efficiency in the Brewing Industry" (26/2/48).

OLIVER, J. H.: "Talk and Film on Matters of Scientific and Social Interest in the Brewing World" (15/1/48).

PREECE, I. A.: "Diastatic Power and the Mash Tun" (9/12/48).

STEWARTSON, D. M.: "Stainless Steel" (29/1/48).

WALKER, T. K.: "Hop Rate, Keeping Quality and Forcing Tray Data" (18/3/48).

An Exhibition by Lt.-Col. F. N. RICHARDSON of New Varieties of Hops, 1947 Growth, was held in the Model Brewhouse, College of Technology, Manchester, on 5th and 6th February, 1948. The Exhibition proved a great success, both technically and socially, being visited by members of the Yorkshire and North-Eastern Section, and by members from over a wide area of this Section.

The Annual General Meeting, followed by a Dinner, was held on 22nd January, 1948, at the Brooklands Hotel, Sale, Cheshire.

The Section has suffered a great loss in the death, after a long illness, on 14th October, of Mr. Rowland WHITAKER, an original Member of the Institute, and the late Managing Director of Groves & Whitnall, Ltd., Manchester; and also of Mr. C. W. W. THORPE, a Director of Greenall, Whitley & Co., Ltd., St. Helens.

YORKSHIRE AND NORTH-EASTERN SECTION

Committee: R. S. Sandbach (*Chairman*), A. J. C. Cosbie, B.Sc., A.R.I.C., A.R.C.Sc.I. (*Vice-Chairman*), E. A. Braithwaite (*Hon. Secretary*), G. L. Dick (*Asst. Secretary and Treasurer*), G. Fielding, B.Sc., R. W. Hill Forster, G. P. Haworth, G. L. Hughes, A. L. Jolliffe, W. Kitchen, E. G. Peet, G. F. Ripley, J. R. Russell, W. J. M. Sproule, A. A. Stewart, N. Tait, C. J. Tayler, W. Waide, and H. Brook.

Mr. A. J. C. COSBIE was nominated to represent the Section on the Council of the Institute in place of Mr. G. A. G. HAWORTH, who has retired from the Committee.

During the year, the following meetings have been held in conjunction with the Incorporated Brewers' Guild:—

BROWN, B. M.: "Research and the Brewer" (18/3/48).

COSBIE, A. J. C.: "American Brewing Methods" (15/1/48). "American Standard Methods of Analysis" (19/11/48).

HAMMERSLEY, R.: "Small Automatic Refrigerating Plants" (16/12/48).

HUNTER, H.: "Some New Varieties in Malting Barley in relation to Northern Requirements" (19/2/48).

LOGAN, R. K.: "Malting and Brewing in New Zealand" (21/10/48).

The meeting on the 19th November, 1948, held at Newcastle-on-Tyne, was the first to be held there since the War.

The Annual Dinner of the Section was held at the Queen Hotel, Harrogate, on the 9th April, 1948.

Luncheons have been held every two months throughout the year, in conjunction with the Yorkshire Section of the Incorporated Brewers' Guild.

Golf. The Section was represented at Little Aston after a lapse of several years, and an Autumn Meeting at Sand Moor was well attended.

MIDLAND COUNTIES SECTION

Committee: B. Wood (*Chairman*), J. A. Lones (*Vice-Chairman*), R. H. Butler (*Hon. Treasurer*), H. J. Cox, A. M. Craven-Smith, H. D. Ensor, G. C. Evans, F.R.I.C., F.C.S., W. Glew, R. L. Hollebone, H. Maynard Mitchell, R. H. Roberts, Ph.D., and J. W. Scott.

Mr. S. H. THOMPSON was nominated to represent the Section on the Council of the Institute. Mr. H. J. COX was nominated to serve on the Examinations Committee.

Six meetings of the Section have been held during the year, at which the following papers were read and discussed:—

CHERRY-DOWNES, H. A. D.: "Mechanization of Maltings" (22/1/48).

FRENCH, E. W.: "Modern Bottling Plant" (19/2/48).

LOGAN, R. K.: "An Outline of the Brewing Industry in New Zealand" (28/10/48).

RICHARDSON, F. N.: "The Season's Hops" (18/11/48).

THOMPSON, H. L.: "The Season's Barley" (30/9/48).

WEIGHTMAN, J. S.: "Modern Public House Equipment" (18/3/48).

The Annual Dinner of the Section was held at the Midland Hotel, Birmingham, on 10th December, 1948.

The Section suffered a great loss through the death of its Hon. Secretary, Mr. Colin K. LANGLEY, on the 26th June. Mr. LANGLEY had been Hon. Secretary since 1935. His partner, Mr. A. G. P. SMITH, M.A., LL.B., has since been appointed as Hon. Secretary.

SCOTTISH SECTION

Committee: L. Fletcher, F.R.I.C. (*Chairman*), A. Clark Doull (*Vice-Chairman*), James Whitton, M.A., B.Com., C.A. (*Hon. Secretary and Treasurer*), J. Morison Inches, J. Speirs, Balfour Thomson, jun., J. M. Wilson, J. H. Melville, A. Davidson, Dr. I. A. Preece, M.Sc., F.R.I.C., M. R. Dewar, J. B. Hamilton-Meikle, D.S.O., J. B. Laing, and Adam Tait.

Mr. Adam TAIT was nominated to represent the Section on the Council of the Institute, and Mr. A. Clark DOULL to serve on the Examinations Committee.

At four meetings of the Section, the following papers were read and discussed:—

BURGESS, A. H.: "Hop Growing" (14/12/48).

SELIGMAN, R.: "The Research Scheme of the Institute—its Past and its Future" (17/2/48).

SKINNER, K. H.: "Scandinavian Brewing Equipment and Methods" (19/10/48).

WATKINS, W. J.: "Pre-Bottling Pasteurization of Beer and Sterile Filling" (20/1/48).

Members of the Section were the guests of the Scottish Section of the Incorporated Brewers' Guild at lectures by Mr. H. L. A. MAY on "The 1948 Season's Hop Crop," and by Dr. I. A. PREECE on "Some Aspects of the Study of Enzymes."

The first post-war Dinner will be held on 18th March, 1949.

BURTON-ON-TRENT SECTION

Committee: J. H. St. Johnston, M.A., F.R.I.C. (*Chairman*), G. C. Matthews, B.Sc., A.R.I.C. (*Vice-Chairman*), A. Slator, Ph.D., D.Sc., F.R.I.C. (*Hon. Treasurer*), A. E. Taylor, A.R.I.C. (*Hon. Secretary*), F. B. Buxton, M.Sc., G. T. Peard, M.Sc., F.R.I.C., E. J. Leeming, M.A., R. C. Sims, A.R.I.C., M. C. Hill, G. H. Williamson, H. S. Atkins, E. S. Deakin, and G. Brown, A.R.I.C.

The Annual General Meeting, followed by an informal dinner, was held on the 23rd January, 1948.

Five meetings of the Section were held during the year, when the following papers were read and discussed:—

BURNS, J. A.: "Methods of Testing Brewery Yeast" (15/10/48).

HOPKINS, R. H.: "Some Recent Developments in the Chemistry of Starch and Diastatic Action" (24/3/48).

HUNTER, H.: "Modern Varieties of Malting Barleys, their Origin and Characteristics" (10/12/48).

PRICE, M. D.: "The Khapra Beetle" (20/2/48).

SHIMWELL, J. L.: "Sources of Bacterial Infection in the Brewery" (12/11/48).

The meeting on the 12th November was held jointly with the Midland Section of the Incorporated Brewers' Guild.

Research

Research Fund Committee: The President and Hon. Treasurer (*ex-officio*), the Chairmen and Vice-Chairmen of the Advisory Sub-Committees (*ex-officio*), R. G. Ault, Ph.D., B.Sc., F.R.I.C., Robert Bruce, Lt.-Col. E. N. Buxton, M.C., G. T. Cook, A. J. C. Cosbie, B.Sc., A.R.C.Sc.I., A.R.I.C., A. Clark Doull, H. W. Harman, Harold Heron, L. H. Lampitt, D.Sc., F.R.I.C., H. R. Lyell, A.K.C., F.R.I.C., W. R. Nicholson, G. T. Peard, M.Sc., F.R.I.C., Colonel J. H. Porter, D.S.O., Robert V. Reid, Walter Scott, B. V. S. Seed, R. Seligman, Ph.D., F.C.G.I., F.I.M., N. B. Smiley, W. T. Smith, W. Stevenson, N. G. Thompson, C. J. Virden, M.A., B.Sc., D.Phil., W. J. Watkins, and Colonel W. H. Whitbread, T.D., B.A. (*Chairman*).

Advisory Research Finance Committee: The President, Hon. Treasurer and Chairman of the Research Fund Committee (*ex-officio*), B. M. Brown, B.Sc., F.R.I.C., G. T. Cook, A. Clark Doull, Colonel J. H. Porter, D.S.O., B. V. S. Seed, N. B. Smiley and W. T. Smith.

The Council regrets to record the retirement of Dr. SELIGMAN from the office of Chairman of the Research Fund Committee, which he had held since 22nd July, 1940.

The following Resolution was passed at a meeting of the Research Fund Committee held on the 16th April, 1948:—

"That the resignation of Dr. Richard SELIGMAN from the Chairmanship of the Research Fund Committee be accepted with very great regret, and that there be placed on record a sense of the Committee's appreciation of his eminent services to the Brewing Industry generally, and the Institute of Brewing in particular, during his tenure of that office."

An illuminated Address, recording this Resolution and signed by all the members of the Committee who had served on the Committee during Dr. SELIGMAN's term of office, was presented, together with an English cut glass crystal rose bowl and two vases, to him by the President at a Meeting of the Council held on the 13th December, 1948.

Colonel W. H. WHITBREAD, T.D., has been elected Chairman of the Committee, in succession to Dr. SELIGMAN.

Colonel J. H. PORTER, D.S.O., and Mr. N. B. SMILEY have been elected Deputy Chairmen.

RECONSTRUCTION OF THE RESEARCH ORGANIZATION

Great progress has taken place during the year under review in the reconstruction of the Research Organization.

The Council has the pleasure to report that Sir Ian HEILBRON, D.S.O., D.Sc., LL.D., F.R.S., to whom reference was made in the last Annual Report, has accepted the position of Director of Research.

The Council has also the pleasure to report that, after prolonged search, a very suitable property, easily accessible to London, has been secured for the purposes of the Research Establishment. This property, Lyttel Hall, Nutfield, near Redhill, Surrey, comprises a fairly modern brick-built residence, so built as to be easily convertible into adequate laboratories, a modern bungalow, a large garage and stable block, with two cottages, together with a single cottage adjacent to a range of farm buildings with loose boxes and a stockyard. The grounds, 37 acres of which are let off to a local farmer, and gardens cover some 55 acres in all.

It is anticipated that the Laboratories will be ready for occupation and use by April, 1950. In this connection, the services of two eminent biologists have been enlisted in order to ensure that the design and equipment of the Laboratories are fully adapted to meet the specialized requirements of modern research.

Pending the opening of Lyttel Hall (to be known as "The Brewing Industry Research Foundation," Nutfield), and the centralization therein of the Institute's research work, arrangements have been made for a large amount of extra-mural work to be carried out, as an interim measure; at the centres, and under the supervision of the gentlemen mentioned below, *viz.*:—

	<i>Researches.</i>
BIRMINGHAM UNIVERSITY Professor M. STACEY.	Work on enzyme systems involved in the carbohydrate synthesis of yeast and bacteria.
CAMBRIDGE UNIVERSITY Professor A. R. TODD, F.R.S.	The nucleosides of yeast.
EDINBURGH UNIVERSITY Professor E. L. HIRST, F.R.S. (assisted by Dr. E. G. V. Percival and Dr. I. A. Preece).	Starches and soluble carbohydrates in barley and malt.
LONDON UNIVERSITY—IMPERIAL COLLEGE Professor Sir Ian HEILBRON, F.R.S.	The chemical constituents of hops and the synthesis of amino-acids and peptides by yeast.
LONDON—ROYAL INSTITUTION Professor E. K. RIDEAL, F.R.S.	The separation of proteins of barley and malt by modern physical methods.
OXFORD UNIVERSITY Professor Sir Cyril HINSHELWOOD, F.R.S.	The systematic study of the influence of mineral salts on the growth of bacteria.
SHEFFIELD UNIVERSITY Professor R. D. HAWORTH, F.R.S.	The tannins of barley, malt and hops.

An immediate start has thus been made on a number of fundamental problems to which answers are urgently required. Furthermore, the active interest and collaboration of eminent scientists have been enlisted in certain aspects of fermentation chemistry which have, hitherto, received insufficient attention in this country.

Another useful aspect of the extra-mural research work now launched in various Universities is that it will ultimately provide the Institute with a nucleus of young scientists with specialized training.

In addition to members of the Institute of Brewing staff now working at Birmingham and Manchester (Dr. R. G. WALLIS, Dr. R. S. W. THORNE and Mr. R. E. ESSERY at Birmingham, and Mr. N. BLAKEBROUGH and Dr. ZAKOMORNY at Manchester), Dr. G. A. HOWARD (now of the Lister Institute), Dr. T. H. FARMER and Dr. G. HARRIS (of Imperial College) and Dr. E. M. SHOOTER (of the Royal Institution) have been, or are being, appointed as members of the permanent staff of Lyttel Hall. Mr. F. G. CONSTERDINE has been appointed as Technical Superintendent of the Laboratories.

Existing contacts with distinguished foreign scientists have been developed by Sir Ian HEILBRON, with a view to close co-operation in the exchange of ideas and, possibly later, of personnel. Among others, mention may be made of Professor LINDERSTRØM-LANG, of the Carlsberg Research Laboratory, Copenhagen, and of Professor TISELIUS, of Uppsala University (the recent Nobel prize-winner); also of Professor WOODWARD, of Harvard University, and of Dr. FRUTON, of Yale, both distinguished for their work on amino-acids and proteins.

Arrangements are being made for Dr. WALLIS to spend some time in the laboratories of Professors SVEDBERG and TISELIUS, at Uppsala University, in order to acquire experience of the most recent techniques of protein separation. It is intended also that Dr. THORNE will spend a few weeks in the Spring in Professor LINDERSTRØM-LANG's laboratory to study the recent micro-methods which have been developed there. Similarly, Dr. FARMER of Imperial College may proceed next Autumn to spend some months with Dr. PONTECORVO, in Glasgow, to learn the fundamentals of genetics and mycological research.

The following Committee has been appointed:—

Directors' Consultative Committee: R. G. Ault, B.Sc., Ph.D., F.R.I.C., L. R. Bishop, M.A., Ph.D., D.Sc., B. M. Brown, B.Sc., F.R.I.C., H. J. Bunker, M.A., A. A. D. Comrie, B.Sc., F.R.I.C., A. Clark Doull, H. Heron, Professor R. H. Hopkins, D.Sc., F.R.I.C., J. H. St. Johnston, M.A., F.R.I.C., B. V. S. Seed, Adam Tait, F.R.I.C., and C. J. Virden, M.A., B.Sc., D.Phil.

Brewers' Society.

The Council is gratified to report that, at a meeting of the Council of the Brewers' Society, it was RESOLVED: "That the furtherance of the research activities of the Institute of Brewing should be a permanent feature of the Society's policy."

COLLEGE OF TECHNOLOGY, MANCHESTER

During the past year, the investigations at Manchester have been prosecuted mainly along the lines which were indicated in last year's report. At the end of February, 1948, Dr. D. KULKA resigned her post on the Institute's Research Staff to take up an appointment on the Staff of the University of Birmingham, and Mr. G. A. THOMAS also left the service of the Institute to take up an industrial post in applied mycology.

Microbiological Investigations. Further study has been made of cases of *Acetobacter* infection in brewery products and particular attention has been devoted to organisms causing ropiness in worts and in beers.

Hop Antiseptics in Beer. Mr. BLAKEBROUGH has continued to study the influence in beer of resin derived from humulone and he is now preparing a report on this subject.

Biological Stability of Beer in Relation to Hop Antiseptic. During 1948, Mr. BLAKEBROUGH undertook further systematic small-scale brewings which had been planned by the Brewing Trials Committee. An interim report on the results has been presented to the Advisory Sub-Committee on Hops, and the latter body has decided that this work should be continued during the current year.

Causes of Loss of P.V. of Hops during Storage. Dr. M. ZAKOMORNY, who was appointed in a temporary capacity to succeed Dr. KULKA, has been engaged upon a study of the chemical changes undergone by humulone in stored hops and he has obtained evidence that a constituent (or constituents) of the β -portion of the soft-resin plays an active part in promoting these losses in storage.

Publications. During 1948, three communications were published in the *Journal*, namely:—

"*Acetobacter* Infection. Part IV. Strains of *Acetobacter suboxydans*, Isolated, respectively, from Pitching Yeast and from Beer." By D. KULKA, J. TOSIC and T. K. WALKER (*J. Inst. Brew.*, 1948, 54, 126).

"A Strain of *Lactobacillus plantarum*, suitable for Use in the Estimation of Antiseptic Power." By D. KULKA and T. K. WALKER (*ibid.*, 1948, 54, 130).

"Capsules of *Acetobacter turbidans* 'Demonstrated' by Positive Staining." By D. KULKA and T. K. WALKER (*ibid.*, 1948, 54, 148).

UNIVERSITY OF BIRMINGHAM

During the year under review, the staff at Birmingham has remained practically unchanged. Considerable delay has been experienced in securing new instruments and replacements, but the work has nevertheless progressed.

Yeast Nutrition Studies. Two papers, "The Growth and Fermentation of Yeast 6479 with Simple Peptides as Nitrogen Nutrients" and "The Bios Requirements of some Top-Fermentation Yeasts in relation to the Nitrogen Source," await publication.

Another paper entitled "A New Mode of Nitrogen Assimilation by Yeast and its Relation to the Problem of Yeast Growth in Wort," has been submitted for publication. This paper offers a demonstration that the principal method of assimilation of nitrogen from beer wort by yeast consists, not in the deamination of amino acids, but in their direct absorption intact, followed by their integration into proteins. Deamination of amino acids by the mechanisms

of Ehrlich and Stickland does occur, but is of secondary importance. This finding provides a resolution of what seemed hitherto paradoxical, namely, that yeast growth in wort is luxuriant whereas it is relatively poor in any of the individual amino acids (where assimilation is necessarily by deamination) which collectively constitute the main part of the assimilable nitrogen of wort.

Work is in progress on (a) the effect of wort original gravity upon the nitrogen requirements of yeast, and (b) nutritional antagonisms between amino acids, *e.g.*, glycine and tyrosine or tryptophane.

Hop Trials. Experimental brewings were carried out, as mentioned elsewhere in this Report, with the two *Verticillium*-resistant varieties, OR 55 and OJ 47.

Wort Composition. As mentioned in the Report for last year, investigations are in progress on the phosphorus compounds of wort and beer, and work on the determination of phytic phosphorus is continuing. In the meantime, methods for the determination of total and inorganic phosphate in wort and beer have been written up. These analyses are not as simple and straightforward as might be supposed. Further investigation of the nitrogen compounds of wort and beer are awaiting the outcome of the corresponding work on barley and malt.

Studies on Barley Proteins. Work carried out in this field has been directed to characterize and establish the degree of purity of some of the protein fractions of barley. The work is divided into two main sections and some time has been spent on the construction of necessary apparatus.

The extraction of both albumin and globulin fractions from Spratt-Archer barley has been carried out, but attention has been mainly focussed on the albumin. Solubility studies made on the albumin have indicated that further fractionation will be necessary; this solubility method will be used to guide the future fractionations needed to give a product of the highest possible purity.

Work has also been carried out on the extraction and purification of the alcohol-soluble protein hordein. It appears from solubility studies that hordein is a reversibly-dissociating system similar to the wheat protein gliadin. It is hoped that an extension of these studies will give much information on the nature of hordein as a reversibly-dissociating system under different experimental conditions.

Some time has been spent on the setting up of hydrogen electrode apparatus which will be used for the study of the combination of various phenolic compounds with egg-albumin and barley proteins. This will form the first part of the work on protein-tannin combination.

Work of a National Character. Reference has been made in previous reports to work carried out in conjunction with the staff of the Low Temperature Research Station, Cambridge, on the concentration of beers, using freezing technique, and their storage and the effects of these on *inter alia* flavour and haze formation. This work has continued.

Vitamins in Beer. Professor HOPKINS and members of the staff of the Brewing School, aided by a grant from the Research Fund Committee, have continued and probably concluded work on this subject. A paper entitled "Pantothenic Acid in Brewing," by Professor HOPKINS, Stella WIENER, Ph.D., and C. RAINBOW, Ph.D., appeared during the year (this *Journal*, 1948, 264).

BARLEY

Grants to the following institutions have been continued, *viz.*—NATIONAL INSTITUTE OF AGRICULTURAL BOTANY, Cambridge (£105) and ROTHAMSTED EXPERIMENTAL STATION, Harpenden (£100).

In the five seasons in question satisfactory crops of "Plumage-Archer" barley of about 6 quarters *per* acre were grown on a heavy loam. The plots that had been dunged for potatoes gave approximately 2 *cwt.* more grain for each 8 tons of dung applied; there was also a similar amount of straw. The major effect of the dung was shown in the potato crop where the increase was about 2½ tons for the single dose, and this is undoubtedly by far the most important result. The further effect shown above in barley though not spectacular was quite a useful supplement.

Treatments applied to sugar-beet gave similar results; the following figures refer to three barley crops in 1945-47:—

Barley.								Cwt. per acre.	
								Grain.	Straw.
Mean Yield	..	..	..	..	..	..	..	32.3	35.8
Increase in barley due to:—									
Deep ploughing for beet	..	..	..	..	..	..	..	+0.7	+0.8
10 tons of dung for beet	..	..	..	..	..	..	..	+1.8	+5.3
1 <i>cwt.</i> muriate of potash for beet	..	..	..	..	..	..	..	+1.0	+3.1

The barley crops in the series were about 8 quarters *per* acre. Where the land was ploughed 12 in. deep for sugar-beet instead of 6 in. deep the effect on the barley was trifling. Dung to beet gave an extra 1.8 *cwt.* of barley grain and 5.3 *cwt.* straw, and 1 *cwt.* muriate of potash applied to beet also gave a small residual effect in barley.

All the evidence points in the same direction, major cultivations and manurings should be applied with a view to their direct effect on the first crop. Of the treatments applied to roots, farmyard manure is the one most likely to carry through to give a further useful benefit to the following barley crop. Whenever there is any doubt whether the land will carry a full crop of corn on the condition built up by previous manuring, farmers always have the means of supplementing this with a direct addition of fertilizer, in which a moderate proportion of nitrogen is the main constituent.

HERIOT-WATT COLLEGE, EDINBURGH

A gift of £500 was made by the Brewers' Society to the Heriot-Watt College in 1925, a condition being that the money should be used for technical purposes in connection with the Brewing Industry. The Governors of the College decided that the capital should be invested and the income allowed to accumulate for the purpose of providing a research scholarship. Mr. A. S. ASHWORTH, B.Sc.Tech., has been appointed for a period of two years, under the said Grant, to carry out an investigation, under the supervision of Dr. I. A. PREECE, on "Cytolysis, and related enzymic changes in germinating barley."

HOPS

THE NEW HOPS RESEARCH INSTITUTE

Reference was made in the last Annual Report to proposals, agreed between the Ministry of Agriculture and the Agricultural Research Council, relating to future arrangements for research into hop production, and to the establishment, in place of the previous *ad hoc* grants for hop research, of a small "Hops Research Institute" at Wye College.

Reference was also made to certain financial arrangements under which the Hops Marketing Board and the Institute of Brewing would share jointly in the capital expenditure of the "Hops Research Institute," entailing the provision of approximately £8,000 by each body, over the next three years. It was also agreed that the annual expenditure (some £6,000) should be met, as to 50 *per cent.*, by the Ministry of Agriculture and, as to the remaining 50 *per cent.*, by the Hops Marketing Board and by the Institute of Brewing in equal shares.

The work carried out during 1948, which follows lines laid down by the Technical Committee on Hop Research of the Agricultural Research Council, was briefly as follows:—

Manurial Trials. Manurial trials in the College hop gardens comparing the effects of organic and inorganic nitrogenous manures and of different quantities of nitrogenous manure, which have been in progress since 1937, were concluded and the results are being examined.

Manurial trial plots have been established in commercial gardens at three centres; these are designed to study the effects of deficiencies of micro-nutrients and of soil acidity upon the yield and quality of the crop. These plots will also give valuable information in connection with the diagnosis of manurial deficiencies in hop gardens.

Cutting the Bine at Picking Time. Investigations on the effect of cutting the bine at picking time have been continued with a view to finding whether this procedure, which must necessarily be adopted when picking machines are used, weakens the plant. These investigations will be carried on for a period of years to find whether any cumulative effect is produced by this treatment.

Selection of Golding Hops. Plants have been propagated from the collection of 120 Goldings which were originally selected from commercial gardens in 1938. These will be planted in a new garden now being established at Wye where they will be tested for cultural characters and brewing value. Analyses of samples of hops from these plants show variation in humulone content within the different varieties.

Deterioration of Hops during Storage. Forty samples of hops, comprising the varieties Early Bird, Fuggle, Brewer's Gold, Bullion Hop, John Ford Hop, and Northern Brewer, obtained with the assistance of the Hops Marketing Board from 22 different growers, were stored at room temperature and the rates of loss of humulone compared. A further trial has been arranged, with hops of the 1948 season's growth, in which storage of similar series of hops at three different temperatures will be compared.

The Hop Nursery. Some 800 plants have been grubbed and replaced with seedlings of diverse parentage.

Green shoots of three varieties—Early Choice, Sunshine Hop and Pride of Kent—of which the supply of sets is insufficient for the demand, were struck in frames in the spring.

Seedlings of *Humulus cordifolius*, raised from seed obtained from Japan, have been planted out and also grown under glass. A representative series of varieties grown commercially in New Zealand has been presented by Mr. A. S. Nash. Rows of the two *Verticillium* Wilt-resistant varieties, OR 55 and OJ 47, have been planted.

Seed has been collected from "open" flowers of selected parents.

Samples of 84 selected new seedlings were picked and dried separately for analysis.

High results for preservative value were obtained with many samples. Among the low results, however, was one with no trace of humulone; this is uniquely valuable for investigation of the brewing value of hops. Some evidence has been obtained of the existence of a "bud sport" of this variety, which will be further investigated next year.

Box samples of nine of the above seedlings were submitted to a merchant and a brewer for examination.

A number of plots of varieties in the Nursery Garden and also in Wye field were picked and dried separately; the majority of these pockets will be used by firms of brewers for brewing tests.

Hop Breeding. Investigations have been made with a view to discovering male hops resistant to *Verticillium* Wilt for use in breeding.

Methods of storing hop pollen to keep it alive for use on early hops in the following season are being studied.

Techniques are being developed for the study of the cytology of both male and female hops.

The relative fertility, *i.e.*, seed production, of commercial and seedling varieties of hops is being compared.

A satisfactory method of applying colchicine to induce polyploidy is being sought.

Verticillium Wilt Investigations. In collaboration with Dr. KEYWORTH, a two-year manurial trial has been laid down at East Malling Research Station. Manurial treatments

were applied in the Spring of 1948 and leaf samples were collected from all the plots on two occasions during the growing season. These samples are now being analyzed to study the uptake of mineral nutrients.

Determination of exchangeable bases of soil samples collected in connection with this investigation were completed and the results have been examined.

Outstation in the Weald. A site in the Fuggle-growing area of the Weald of Kent has been selected for an outstation to test the suitability of the new varieties for that district. Negotiations are in progress for leasing the land.

Experimental Kilns at Beltring. These kilns were destroyed by fire in 1947. Negotiations with the Insurance Company were made and the claim for their loss has now been settled.

Advisory Work. During the year 57 visits have been made to hop farms to advise on soil problems and manuring. Forty visits have also been made to oast houses to advise on alterations and reconstruction and to diagnose and remedy troubles arising during use.

Advice has also been given by correspondence and to many growers who have called at the College to discuss problems of oast house construction and management, soils, manuring and other matters concerning the industry.

A paper on "Hop Growing" was read to the Scottish Section of the Institute in December.

EAST MALLING RESEARCH STATION

REPORT ON COMMERCIAL VARIETIES

(a) *Goldings.*

Detailed records have been continued on the seven clonal Golding selections. With the co-operation of the Institute of Brewing it has been possible to arrange for large-scale and Laboratory brewing trials with the 1948 growths.

(b) *Fuggles.*

Some 100 individual Fuggle clones have been maintained. Cuttings from a few of the more promising ones will be planted at the Wye centre and form the basis of a new Fuggle strain trial.

Propagation Trials.

Trials designed to measure varietal and seasonal differences in the take of strap-cuts in the New Varieties and the clonal Goldings have been concluded. A report on the complete series of trials will be prepared when the data for the past season have been statistically examined.

Other Investigations.

Further information on the amount of pull required to detach the cones of the principal varieties has been outlined. An account of the three years' observations is being prepared.

REPORT ON DISEASES OF HOPS

Verticillium Wilt.

(a) *Survey.*

Partly as a result of the introduction of the *Verticillium* Wilt of Hops Order (1947), requiring notification of all cases of progressive *Verticillium* Wilt to the Ministry of Agriculture, some 23 outbreaks of progressive Wilt which had previously not been recorded by the Research Station were reported by officers of the National Agricultural Advisory Service during 1948. Of these, probably six were of recent origin, the others being of from two to six years' standing. One progressive outbreak was reported from West Kent and another, which was possibly progressive, from North Kent, both being some miles from the nearest previously reported outbreak.

Fluctuating Wilt was particularly prevalent and caused considerable wilting in many gardens, both in Kent and Sussex and in the West Midlands. The severity of the symptoms

was presumably correlated with the high rainfall and low temperatures prevailing during the growing season. Two such cases in the West Midlands were apparently associated with the prior planting of potatoes.

(b) *Resistance Trials.*

One new resistant variety was selected from the Wye Seedlings tested during 1947. No variety has yet been found more suitable for commercial use than OR 55 and OJ 47, the two previously selected as the best for this purpose. Further tests were made of the progeny of known resistant varieties and several were selected for propagation. The breeding of new Wilt-resistant varieties at East Malling has now been concluded in view of the start of such work at Wye College, but the testing of the varieties which have already been bred will be continued.

Propagation of the resistant varieties by new layering techniques has been extended and advice given on the establishment of large-scale nurseries for this purpose by the Hops Marketing Board and a commercial firm. Accounts of the methods are in the course of publication.

(c) *Studies of fluctuating and progressive outbreaks.*

An account of the earlier work on this subject has now been published in which the methods used to determine the relative pathogenicity of isolates of *Verticillium albo-atrum* from fluctuating and from progressive outbreaks are described. Isolates from fluctuating outbreaks were less pathogenic to young hop plants than those from progressive outbreaks, and it is suggested that this is the main cause of the differences in symptom incidence and intensity between the two types of outbreak in the field.

A note has also been published on the incidence of symptoms of fluctuating wilt in early, mid-season and late bine. It was found that late dressing and training of the plants reduced the incidence of the "fat-bine" symptom. Another publication now in the press describes a study of the occurrence of the fungus in cuttings from plants in a garden affected by fluctuating wilt.

Further work has been undertaken on the differentiation of the strains of *V. albo-atrum* by laboratory cultural methods.

(d) *Wilt incidence in relation to the nutrition of the plant.*

An account is now being published of the collaborative experiment on this subject undertaken at Long Ashton Research Station in which clear correlations were observed between wilt incidence and the nitrogen status of the plants. The field experiments on this problem have been continued and a further trial laid down.

Virus and suspected virus diseases.

(a) *Nettlehead.*

An account has been published of recent work on the graft-testing of certain Wye Seedling varieties for sensitivity to this disease in which the eighteen varieties were found to show symptoms; also on the results of aerial surveys and subsequent ground mapping of outbreaks of Nettlehead disease. A preponderance of plants showing symptoms was found on the sites of old hedgerows in certain fields. In another field there were many more affected plants on a part that had formerly been old pasture than on the formerly arable section. No evidence was obtained on the basic causes of the effects observed. These correlations have been further studied and experiments planned to test various hypotheses advanced to explain them.

Observations on symptom masking have again been made in commercial gardens. During a study of symptoms, enations were noted on the leaves of an infected plant in the greenhouse. Subsequent observations in the field showed that in most Nettlehead outbreaks some plants bore enations, but that the symptom could not be relied upon in diagnosis because many plants which were definitely infected did not show it. Enations were not found on healthy plants.

(b) Split Leaf Blotch.

Observations were made on infected plants in commercial gardens and symptoms recorded again on the plants used in the graft transmission experiments made in 1946.

(c) Other disorders.

Observations were made on some of the plants on which symptoms of "Leaf Curl" had been seen in 1947. The "Leaf Curl" symptom was not often apparent this year, but some leaves on the plants bore necrotic flecks similar to those noted in 1947. Preliminary grafting experiments were made with material collected from the plants. Further observations were made in several localities of other leaf symptoms of various types and cuttings were collected for further study.

(d) Virus vector tests.

The intensive study of the vectors of hop virus diseases was started in November, 1947, the first season's work being mainly of an exploratory nature.

The hop-damson aphid, *Phorodon humuli* Schr., was tested as a possible vector of Nettlehead and Mosaic diseases, using plants of the Fuggle and Eastwell Golding varieties housed in temporary greenhouses. Long feeding periods were used to test for persistence of the virus, and starvation for one hour, followed by short feeding periods for non-persistence. The number of aphides employed varied between five and 200 per test plant. No symptoms of the diseases were seen during the growing season on the test plants, which will be planted out for further observations during 1949.

Pilot experiments were carried out using hop red spider, *Tetranychus telarius* L., on mosaic, and flea-beetle, *Psylloides attenuata* Koch, on Nettlehead. No symptoms of the diseases were found on the test plants during the growing season of 1948. These plants also will be planted out for observation during 1949, when further trials will be made with the same insects and other common insects which feed on hops.

NEW VARIETIES OF HOPS

Conference. A conference was held, under the auspices of the Hops Marketing Board, on the 4th November, 1948, for the purpose of considering *inter alia* (a) the question of the suitability of certain new varieties, specified below, for brewing; (b) the question of increasing, restricting or discontinuing any of such varieties; and (c) the question of their commercial valuation. These hops were:—

Brewers' Gold
Bullion Hop
Early Promise
Early Choice
Northern Brewer
John Ford

Concord Hop
Pride of Kent
Sunshine
Malling Midseason, and
Quality Hop.

A number of suggestions arose in the discussion which ensued, and Mr. B. M. BROWN and Mr. G. T. COOK have been nominated to represent the Institute on a Committee appointed by the Board to consider them. The conference was, however, unanimous that new varieties should not be grown at the expense of Goldings.

Challenge Cups. The annual exhibition of New Varieties of Hops, which was held in the showrooms of Messrs. WIGAN, RICHARDSON & Co., London, on the 27th and 28th January, 1948, was visited on each day by large numbers of brewers and growers.

The competition was again divided into two classes, that is to say, Class "A" (to augment English Commercial Varieties), and Class "B" (Substitutes for American Hops).

The Growers' Cup was presented by growers of new varieties for the best sample in Class "A," and the Brewers' Cup, presented by Barclay Perkins & Co., Ltd., Arthur Guinness, Son & Co., Ltd., and Whitbread & Co., Ltd., was awarded for the best sample in Class "B."

Both cups were gained by Mr. J. A. WORLEY, of Chart Sutton, Mid-Kent. His Class "A" entry was a sample of Brewers' Gold (C9a), and his Class "B" entry was a sample of Bullion (Q43).

An exhibition of these hops was subsequently held, by kind permission of Professor KENNER, in the College of Technology, Manchester, on the 5th and 6th February, 1948.

With regard to the forthcoming competition, that is to say, in respect of the hops of the 1948 growth, for which some 32 samples have been entered, it has been decided that entries in Class "A" should be hops with aromas which, in the opinion of the judges, are similar to those usually associated with English hops.

It has also been decided that growers need not suggest the class into which their particular exhibits should be placed, and that this matter might well be left to the discretion of the judges.

Exhibitions will again be held in London and Manchester, and also in Edinburgh.

THE GOLDING HOP

One acre of land at the East Malling Research Station is devoted to the experimental cultivation of clonal varieties of the Golding hop. The following are now in their fifth year of cultivation, *viz.*:—Bramblings, Canterbury Goldings, Early Birds, Eastwell Goldings, Mathons, Petham Goldings and Rodmershams.

Brewing trials of these hops will be carried out by Fremfords, Ltd., Maidstone.

VERTICILLIUM WILT

Brewing trials have been carried out in the small-scale brewing plant at the University of Birmingham, at the instance of the Hops Marketing Board, with samples of two new varieties, namely OR55 and OJ47, which were considered to be resistant to *Verticillium* Wilt.

All the judges preferred OR55 to OJ47, although neither could be brewed with alone. The opinion was indeed expressed that the unpleasant bitter flavour of OJ47 would make this hop unacceptable, as a commercial possibility, even in a blend. On the other hand, OR55, although producing a stronger bitter flavour than the control (a Fuggle hop), was considered to be capable of use in a blend provided that it represented not more than 25 *per cent.* of the total hops used.

These results were confirmed by two large London breweries which also carried out trials of these hops.

The Hops Marketing Board has, however, cultivated a small number of sets of these two varieties during the current season, thus making possible trials on a somewhat larger scale than hitherto.

THE INSTITUTE OF BREWING YEAST COLLECTION

Arising out of the British Commonwealth Specialist Conference held in London, under Government auspices, in August, 1947, the Institute has agreed to make itself responsible for the Commonwealth Culture Collection of Yeasts which formed part of the National Type Culture Collection hitherto maintained in this country under the auspices of the Medical Research Council.

In this connection, the Institute is indebted to Mr. H. J. BUNKER, who so kindly received the cultures (some 200 in all) on behalf of the Institute, and, in addition, distributed them amongst a number of firms, mentioned below, which have generously agreed to maintain them, and, moreover, to supply strains to those requiring them pending the housing of the collection at the Institute's Research Establishment, *viz.*:—

Barclay Perkins & Co., Ltd.
Bass, Ratcliff & Gretton, Ltd.
Charrington & Co., Ltd.
Distillers Co., Ltd.

A. Guinness, Son & Co., Ltd.
Mitchells & Butlers, Ltd.
Whitbread & Co., Ltd.
Wm. Younger & Co., Ltd.

A card index of the cultures will be kept at the office of the Institute.

A list of the cultures and particulars of the arrangements made for the conduct of the collection and for the distribution of strains will appear in the January/February (1949) Number of the *Journal*.

ACKNOWLEDGEMENTS

The Council desires to express its thanks to the many members who have served the Institute in various ways during the year, and especially the members of committees and the local representatives.

ACCOUNTS

The accounts of the Institute for the year under review, which have been audited by Messrs. THOMAS & Co., are appended.

By Order of the Council,

W. H. BIRD,

Secretary.

4th March, 1949.

THE INSTITUTE

INCOME AND EXPENDITURE ACCOUNT (GENERAL FUND)

1947		EXPENDITURE.	£	s.	d.	£	s.	d.
£	s.	d.						
		To Cost of Institute's Journal:						
736	16	5	Composing, Printing, etc.			883	7	6
713	0	8	Printing Advertisements			782	17	11
44	16	3	Reprints			145	12	9
153	4	7	Postage and Despatch			173	13	7
83	12	9	Abstractors, Reviewers, etc.			80	7	10
798	16	5	Editor and Assistant Editor: Salaries and Expenses			799	19	2
16	13	7	Sundry Expenses			25	1	3
							2,891	0 0
2,547	0	8						
25	0	0	" COST OF COLLECTIVE INDEX				25	0 0
134	15	3	" EXAMINATION EXPENSES				185	18 8
106	0	2	" MEETING EXPENSES				108	10 1
			" OFFICE EXPENSES:					
292	2	6	Rent, Cleaning, etc.			287	12	6
2,921	0	0	Salaries			3,498	0	0
116	7	11	Stationery and Printing			164	6	10
153	6	4	Stamps, Telephone, etc.			139	15	0
58	13	1	Petty Cash Expenses			53	6	7
							4,143	0 11
			" SUNDRY AND SPECIAL EXPENSES:					
79	17	4	Income Tax on Investments			85	0	0
139	7	8	Audit, Insurance, Secretary's Expenses, Travelling, etc.			232	18	2
81	4	1	Subscriptions, etc., written off			65	2	0
70	12	3	Depreciation on Furniture			60	0	5
			"Standard Methods of Malt Analysis" (Reprinting)			36	18	6
126	18	1	Sundry Expenses			65	18	3
210	13	4	List of Members, 1947—Printing and Despatch					
339	0	3	Banquet, 1947 (net Cost)					
							545	17 4
482	12	4	" GRANTS TO SECTIONS				584	5 2
7,884	11	3	Total Expenditure				8,483	12 2
5	13	5	Excess of Income over Expenditure (General Fund)				1,179	19 3
£7,890	4	8					£9,663	11 5

BALANCE SHEET (GENERAL

1947		LIABILITIES.	£	s.	d.	£	s.	d.
£	s.	d.						
519	19	10	SUNDRY CREDITORS AND CREDIT BALANCES			463	10	5
50	0	0	COLLECTIVE INDEX RESERVE			75	0	0
156	5	0	MEMBERS' SUBSCRIPTIONS received in advance			93	0	0
122	5	8	SUBSCRIPTIONS TO JOURNAL received in advance			121	15	4
							753	5 9
848	10	6						
5,000	0	0	JOHN S. FORD MEMORIAL TRUST FUND			5,000	0	0
65	2	4	Add Net Income to 31st December, 1947			65	2	4
			Add Interest on Investments and Tax Refunds			5,065	2	4
						179	19	11
			Less Award and Expenses, 1948			5,245	2	3
						149	0	0
							5,096	2 3
			BALANCE IN FAVOUR OF INSTITUTE GENERAL FUND:					
8,791	16	9	On 31st December, 1947			8,797	10	2
5	13	5	Add Excess of Income over Expenditure, 1948			1,179	19	3
							9,977	9 5

REPORT OF THE AUDITORS TO THE MEMBERS OF THE INSTITUTE OF BREWING (GENERAL FUND)

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for the purposes of our audit. In our opinion proper books of accounts have been kept by the General Fund so far as appears from our examination of those books. We have examined the above Balance Sheet and annexed Income and Expenditure Account, which are in agreement with the books of account. In our opinion and to the best of our information and according to the explanations given us the Balance Sheet gives a true and fair view of the state of affairs of the General Fund as at 31st December, 1948, and the Income and Expenditure Account gives a true and fair view of the result for the year ended on that date.

10th March, 1949.

THOMAS & Co., Incorporated Accountants, Auditors,
Manfield House, 378-9, Strand, London, W.C.2.

£14,711 3 0

£15,826 17 5

1st January to 31st December, 1948.

£7,890 4 8

£9,663 11 5

1947			ASSETS.			1948			1949		
£	s.	d.				£	s.	d.	£	s.	d.
271	0	1	CASH AT BANK (CURRENT ACCOUNT)	..	..	171	7	0			
2,450	0	0	" " (DEPOSIT ACCOUNT)	..	..	1,700	0	0			
19	16	1	" OFFICE	..	..	23	7	10			
									1,894	14	10
INVESTMENTS:											
			£3,000 3½ per cent. War Stock, 1952, at cost (less £20 19s. 2d.								
2,890	1	11	Conversion Bonus)	..	..	2,890	1	11			
326	9	9	£350 Conversion 3½ per cent. Stock, at cost	..	..	326	9	9			
679	7	0	£650	..	..	679	7	0			
-	-	-	£1,953 4s. 3d. 2½ per cent. "National War" Bonds (1952-54) at	..	..						
			cost	..	..	2,000	0	0			
992	14	3	£1,000 Conversion Loan 3 per cent. (1948-53) at cost	..	..	-	-	-			
			(Market Value of Investments: £6,181 0s. 0d.)							5,895	18
JOHN S. FORD MEMORIAL TRUST FUND: INVESTMENTS:											
5,000	0	0	£4,800 Redemption Stock 3 per cent. (1986-96)	..	..	5,000	0	0			
-	-	-	Cash at Bank	..	..	31	6	3			
65	2	4	Income Tax refunds due	..	..	64	16	0			
									5,096	2	3
400	2	7	FURNITURE, less Depreciation	..	..				340	2	2
SUNDRY DEBTORS AND DEBIT BALANCES:											
889	3	10	Advertisers	..	..	953	13	4			
78	9	5	In hands of Sections	..	..	49	10	8			
52	10	0	Members' Subscriptions in Arrear	..	..	78	5	0			
			Due from Research Fund Account:								
289	14	0	Establishment Charges	..	..	1,211	1	4			
306	11	9	Sundry Debit Balances	..	..	307	9	2			
									2,599	19	6
									£15,826	17	5
£14,711 3 0											

THE INSTITUTE

INCOME AND EXPENDITURE

1st January to

1947			EXPENDITURE.	£	s.	d.	£	s.	d.
£	s.	d.							
			To RESEARCH DIRECTOR'S ESTABLISHMENT:						1,947 7 10
449	10	11	Salaries, Superannuation and Expenses						
			.. INSTITUTE RESEARCH LABORATORIES, BIRMINGHAM:						
2,969	15	2	Salaries, Wages and Superannuation	4,281	14	4			
380	14	0	Chemicals and Apparatus	1,359	13	9			
115	15	7	Sundry Expenses (Travelling, Stationery, Post, etc.) ..	180	0	3			
							5,821	8	4
			.. HOP INVESTIGATIONS (KENT):						2,704 15 0
370	13	2	Grant to Hop Research Station, Wye						
			.. HOP INVESTIGATIONS (MANCHESTER):						
1,733	6	8	Salaries and Superannuation	1,497	1	8			
297	8	6	College Fees, Apparatus, Chemicals and Sundries ..	289	19	8			
							1,787	1	4
			.. BARLEY INVESTIGATIONS:						
100	0	0	Rothamsted Experimental Station: Grant	100	0	0			
105	0	0	National Institute of Agricultural Botany: Grant ..	105	0	0			
							205	0	0
			.. EXTRA-MURAL RESEARCH:						
-	-	-	University of Birmingham	366	13	4			
-	-	-	University of Edinburgh	248	6	8			
-	-	-	Imperial College of Science, London	360	0	0			
-	-	-	Royal Institution, London	101	16	7			
250	0	0	Dr. J. L. Shimwell: Research on "Ropiness in Beer" ..	250	0	0			
							1,326	16	7
			.. LYTTEL HALL, NUTFIELD:						
-	-	-	Upkeep and Management Expenses	107	2	0			
-	-	-	Legal Expenses incidental to purchase	465	18	1			
-	-	-	Sundry Expenses ditto	359	10	6			
							932	10	7
			.. SUNDRY EXPENSES:						
602	11	0	Income Tax	1,067	10	10			
1,789	14	0	Institute Charges under Bye-Law 93	3,211	1	4			
225	16	2	Reports, Printing and Stationery	287	14	4			
614	5	7	Sundry Expenses (Subscriptions, Audit, Insurance, etc.)	761	4	3			
							5,327	10	9
10,004	10	9	Total Expenditure				20,052	10	5
51,699	3	9	.. Excess of Income over Expenditure (Research Fund)				42,665	10	1
							£62,718	0	6

BALANCE SHEET

31st December,

1947			LIABILITIES.	£	s.	d.	£	s.	d.
£	s.	d.							
566	12	7	SUNDRY CREDITORS	1,116	9	10			
			Do. Due to Institute General Fund for						
289	14	0	Establishment Charges	1,211	1	4			
50	0	0	COLLECTIVE INDEX RESERVE	75	0	0			
							2,402	11	2
			BALANCE IN FAVOUR OF INSTITUTE RESEARCH FUND:						
49,418	11	6	On 31st December, 1947	101,117	15	3			
-	-	-	Add Surplus realized on Experimental Kilns, Beltring,						
			destroyed by fire	893	4	3			
				102,010	19	6			
51,699	3	9	Add Excess of Income over Expenditure, 1948 ..	42,665	10	1			
							144,676	9	7

REPORT OF THE AUDITORS TO THE MEMBERS OF THE INSTITUTE OF BREWING (RESEARCH FUND)

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for the purposes of our audit. In our opinion proper books of accounts have been kept by the Research Fund so far as appears from our examination of those books. We have examined the above Balance Sheet and annexed Income and Expenditure Account, which are in agreement with the books of account. In our opinion and to the best of our information and according to the explanations given us the Balance Sheet gives a true and fair view of the state of affairs of the Research Fund as at 31st December, 1948, and the Income and Expenditure Account gives a true and fair view of the result for the year ended on that date.

10th March, 1949.

THOMAS & Co., Incorporated Accountants, Auditors,
Manfield House, 376-9, Strand, London, W.C.2.

£102,024 1 10

£147,079 0 9

OF BREWING

ACCOUNT (RESEARCH FUND).

31st December, 1948.

1947												
£	s.	d.	INCOME							£	s.	d.
60,000	0	0	By BREWERS' SOCIETY: Allocation	..	..	..	..	..	..	60,000	0	0
288	16	0	„ SUBSCRIPTIONS..	..	..	..	..	..	..	288	16	0
1,395	1	2	„ INTEREST FROM INVESTMENTS, GROSS	..	..	..	..	..	..	2,351	9	0
16	14	4	„ SUNDRY RECEIPTS	..	..	..	..	..	..	67	15	6
3	3	0	„ Donations	..	..	..	..	..	..	-	-	-

£61,703 14 6

£62,718 0 6

(RESEARCH FUND).

1948.

1947															
£	s.	d.	ASSETS.							£	s.	d.	£	s.	d.
			CASH AT BANK:												
347	18	0	On Current Account	..	..	..	..	..	..	486	12	2			
19,500	0	0	On Deposit Account	..	..	..	..	..	..	5,000	0	0			
12	16	0	CASH AT OFFICE	..	..	..	..	..	..	11	14	9			
													5,498	6	11
			INVESTMENTS:												
5,620	7	9	£7,500 Conversion Loan (3½ per cent.), at cost	..	..	..	..	..	..	5,620	7	9			
			£11,000 (3½ per cent.) War Stock, 1952, at cost, less £90												
10,955	13	5	Conversion Bonus	..	..	..	..	..	..	10,955	13	5			
4,337	16	0	£5,000 (4 per cent.) Funding Stock, 1960-90, at cost	..	..	..	..	..	..	4,337	16	0			
5,000	0	0	£5,000 (3 per cent.) Savings Bonds, 1955-65	..	..	..	..	..	..	5,000	0	0			
1,000	0	0	£1,000 (3 per cent.) Defence Bonds	..	..	..	..	..	..	1,000	0	0			
1,500	0	0	£1,500 (2½ per cent.) National War Bonds, 1951-53	..	..	..	..	..	..	1,500	0	0			
-	-	-	£2,929 16s. 4d. (2½ per cent.) ditto 1952-54, at cost	..	..	..	..	..	..	3,000	0	0			
50,432	18	9	£65,000 (1½ per cent.) Exchequer Bonds, 1950, at cost	..	..	..	..	..	..	65,518	11	9			
-	-	-	£12,205 17s. (2½ per cent.) Nat. War Bonds, 1952-54, at cost	..	..	..	..	..	..	12,500	0	0			
2,968	8	6	£3,000 Conversion Loan (3 per cent.) 1948-53, at cost	..	..	..	..	..	..	-	-	-			
			(Market Value of Investments: £113,937 0s. 0d).										109,432	8	11
-	-	-	FREEHOLD PROPERTY (Lyttel Hall, Nutfield), at cost, including Development Charge	..	..	..	..	..	..				15,500	0	0
-	-	-	FIXTURES AND FITTINGS, at cost	..	..	..	..	..	..				100	5	0
-	-	-	LABORATORY APPARATUS, EQUIPMENT AND LIBRARY, at cost	..	..	..	..	..	..				510	8	8
25	0	0	Hop Kilns, Beltring: at cost (less written off)	..	..	..	..	..	..				-	-	-
323	3	5	SUNDRY DEBTORS AND DEBIT BALANCES	..	..	..	..	..	..				16,037	11	3
													£147,079	0	9

MEETING OF THE MIDLAND COUNTIES' SECTION HELD AT THE WHITE HORSE HOTEL, CONGREVE STREET, BIRMINGHAM, ON THURSDAY, 18TH NOVEMBER, 1948.

MR. B. WOOD in the Chair

The following paper was read and discussed:

THE SEASON'S HOPS, 1948

By F. N. RICHARDSON

THE 1948 growing season was in general a wet one with temperatures below average and too many cold nights. Wet conditions early on are not harmful to Fuggles which comprise about 75 *per cent.* of the crop, provided that they do not continue into August. The Golding prefers a hot dry summer. At one time it looked as if August might give some sunshine and warm nights but the promise failed to mature. Except in the *Verticillium* wilt area of Mid Kent, disease was not as troublesome as a poor summer would lead one to expect. Aphis and mould were kept under control by regular spraying or powdering, and downy mildew, which in the wet conditions was likely to spread, was not allowed to become dangerous, although some samples show traces of it.

In the earlier part of the season the gardens suffered from constant, strong winds, which had the effect of making powdering and spraying a good deal less effective than they should have been. Heavy rains during the period of pollination tended to prevent the female plant from being fertilized by the male, and this probably accounts for the fact that the hops this season have fewer and smaller seeds than usual. In the third week of August there appeared to be a very large crop on the bine, which without being at all heavy, was sufficient to carry it, but the final weather conditions did not allow the hops to fulfil their promise. Thus, the quality of the crop can only be described as average. It is more even than in 1947, with fewer choice samples at one end and less "tails" at the other. The P.V. may be expected to be generally considerably lower than last season, but as yet enough figures have not been seen to form a reliable opinion.

It is not a Golding year. Golding growths are down over 30 *per cent.* and this is a serious matter when under normal conditions the demand for Goldings greatly exceeds the

supply. In East Kent the quality of the Goldings is only fair with some good samples, and the few Mid Kent Goldings are disappointing. Worcester on the other hand has produced some very good Goldings, both Bramblings and Mathons. As regards Fuggles, the best will probably come from the Weald of Kent and from the Midland Counties. Some of those from the latter district look very nice indeed and have a good full aroma, which is pleasing after last year's disaster. It is said that Farnhams will be some of the best hops this season. Fuggles from Mid Kent will probably be of ordinary quality, whilst those seen from Sussex are in many cases definitely poor.

On 4th November a Conference was held in London on the question of the new varieties of hops and the Marketing Board invited to it representatives of all interested parties. These new varieties have only been available to brewers during a period of shortage, and it has therefore been impossible to find out whether the demand for them was genuinely as new varieties, or whether they were merely wanted as hops. Now the position of shortage is ended and brewers are asked to say which varieties they wish continued as commercial growths. Some twelve are being grown commercially, and in 1947 a few thousand pockets were produced. The writer's opinion is that Bullion and Brewers' Gold (which can be called substitutes for American hops) should be grown, but he has not so far seen any new variety likely to compete with the established English commercial varieties. The best of this competitive class are Northern Brewer and John Ford, which should continue in production. It is also very important to continue trying to produce a variety really resistant to *Verticillium* wilt. OR55 and OJ47 are reputed to be so, but more experience of them is required.

The acreage under hops to-day is 22,700

statute acres, and the statute acre has now finally been accepted by all parties as the official measure. The old "hop acre" was not a measure of area in the strict sense, being determined by the number of hills in an area, but as growers have their lanes of different widths and their hills at distances apart which may vary from farm to farm, this method of computation was unreliable for statistical purposes. The statute acre is the Ordnance Survey measure of the farm devoted to hops including the headlands, *etc.*, necessary for turning tractors.

Just after the war, brewers asked for and obtained an increase in acreage. Their request was reasonable at the time, and the Hops Marketing Board as well as the Ministry of Agriculture encouraged planting. Unfortunately no one foresaw two Budget increases of 1*d.* per pint and a 3 *per cent.* drop in permitted barrelage on account of sugar shortage. To-day 22,700 acres is probably in excess of the brewers' needs.

Ignoring the production of brewer growers and certain contracts, the number of pockets consigned to the Hops Marketing Board is 167,736, which at 1½ *cwt.* net *per* pocket gives a net figure for the crop of 251,604 *cwt.*, against 268,000 in 1947. In mid August the writer forecast a crop of over 300,000 *cwt.*, and the hops were undoubtedly there, but they did not grow out and developed a lower seed content. The latter is of course an advantage to the brewer. It usually takes about 140 bushels of green hops to make one dried pocket; this year it took 170 to 180.

The estimated market demand submitted by brewers last May totalled 278,000 *cwt.*, of which 99 *per cent.* or about 275,000 *cwt.* was contracted for firm. The Board has had to make an allowance for all-fault hops, which this season for the first time since 1938 do not form part of the contract, and also to hold back in reserve enough hops to provide replacements for hops rejected under Clause 11 of the contract. The former amount to 3,500 pockets and the latter 3,000. These provisions permit of a release of 88 *per cent.* which was announced on 28th October. Thus, all contracting brewers will receive 88 *per cent.* of their firm contract quantity, or 88 *per cent.* of each merchant's nominated quantity. Sometime in the Spring there will in all probability have to be a further small percentage taken up by all contracting

brewers in order to absorb any merchantable surplus in the Board's reserve which is not subject to Clause 11 of the contract.

All-fault hops this year may total over 3,000 pockets against 995 last year and between 800 and 1,000 in most years. Brewers are no longer obliged to accept these as part of their contract, and this season only just over 300 pockets have so far been taken as additional hops. In view of this absence of demand the Board has put some of these pockets into cold store, and others have been sent back to the farms for re-drying or were re-dried before leaving the farms. The Board has agreed that these re-dried pockets shall be valued eventually in separate grades from the rest of the growth, but there is an important point to which it so far will not agree. When these re-dried pockets return to warehouse the Board contends that they are sound packages and must be accepted within the contract quantity. The writer would submit, however, that once having been categorized as "all-faults" nothing in the way of processing can alter that category. In order to re-dry them the packages have been interfered with and at best they should only be described as "repacks." The hop grower to-day is being paid by the brewer a very fair price indeed for his hops; in fact the writer is convinced that last year's average price of £23 10*s.* was not justified. In view of this price level there would appear to be no reason why the brewer should be forced to accept as part of his contract an inefficiently produced and defective article.

The fixing of the average price is now-a-days difficult. The Ministry of Agriculture just after the war arranged for Wye College and Bristol University to investigate annually the costings of hop growing in the South-Eastern and Midland Counties respectively. These figures are submitted to the Permanent Joint Hops Committee and argued before this Committee by the accountancy experts of The Brewers' Society and the Hops Marketing Board. The final decision lies with the three independent members of the Committee. One of these is an eminent accountant who has been investigating the whole question. His report is expected shortly and it is hoped thus to arrive annually at a figure fair to both sides. Until he makes his report on the question in general and the season's costings have been received from

the two colleges, it is unwise to try to forecast what the average price for this crop is likely to be.

As a result of the guarantee given by brewers to growers not to import any hops from abroad provided the English product is sufficient and of reasonable quality—with the one exception of up to 1,000 *cwt.* of hops for lager brewers—the quantity likely to be imported this year is only about 500 *cwt.* for lager beer. Any imports are subject to the permission of the Board of Trade, the Treasury and the Ministry of Food, but the existing restriction of imports is really a voluntary one on the part of the brewing trade in an attempt to give real assistance to English growers.

Some explanation may be given about the impossibility of returning yet to the distribution procedure of the immediate pre-war years and the continued necessity of the Distribution Scheme. The whole obstacle is warehouse accommodation. In the 1940 blitz on London the hop trade lost two-thirds of its warehouse accommodation, and is left with capacity for only 50,000 pockets. The South-Eastern Counties crop totals more than double this, requiring therefore a double put-through. As soon as war ended the hop merchants combined to form a company to build a large communal warehouse in the Borough to hold 40,000 pockets. The Company exists and its directors, of which the writer is one, have worked ceaselessly on the project, but so far have been unable even to buy a site in a satisfactory location. The town planning situation in South London is such that it cannot yet be predicted when a start can be made. Even if a site were obtainable, construction would certainly not be allowed at present. Meantime the distribution scheme works, even if it by no means satisfies either the merchants or their customers, but the very fact that it has been made to work by all parties lowers the priority for a new warehouse.

A very important statement must be made about pocketing. Owing to the situation between India and Pakistan, the supply of jute is even smaller than in recent years. Trade relations between these two Dominions are not good and an exchange of jute from Pakistan for coal from India has broken down. Jute is grown in Pakistan which has no mills and milled in India which grows none. As a result, the jute controller—who

last season allocated enough jute to make 80 *per cent.* of the new pockets required—has now asked those concerned to find 50 *per cent.* of the pocketing for the 1949 crop from used pockets. This is not possible. The requirement for 1949 is estimated at 220,000 pockets and merchants have offered as a maximum to re-condition 40 *per cent.* or 88,000 pockets, but this can only be done if brewers will return all the cloths they can, and in the best possible condition. All brewers are, therefore, asked to ensure that when each pocket is opened only the string is cut and not the selvidge through which the string passes. If the selvidge is cut, the cloth is either unfit for re-use or considerable patching is necessary. It is very much in every brewer's future interest that great care is taken when pockets are opened, and it may be suggested that one of the under-brewers should be made "pocket officer" in the same way that many breweries have appointed a "fuel officer."

The question of getting the hop crop picked within a reasonable time is worrying many growers, especially the larger ones. Labour for hand picking is still available, but the old-time free and easy holiday atmosphere has gone and pickers are not easy to handle. The cost used to be 4 or 5 bushels *per* shilling, but now runs at 1s. to 1s. 3d. *per* bushel. Several growers have installed machines. These are expensive—costing about £10,000 to £12,000—and cumbersome. Their use is confined to 2 to 3 weeks a year and depreciation is heavy. Furthermore, no machine so far produced has managed to avoid shattering the hops. There is no doubt that, as in America to-day, so in this country before long, machine picking will displace the hand method, but a much improved machine must be produced if good results are to be obtained. There are two kinds of machine, the static and the portable. The former is the one more suited to England and is housed in a large shed. The latter, which moves behind a tractor through the garden, is unsuited to this country where the lanes are too narrow, the garden too small, and the ground in harvest time often too wet to avoid bogging down.

In view of the large acreage and the probability of surpluses in the coming years, merchants are trying very hard to open up export markets. The main obstacle is the fact that most overseas breweries make a lager type beer for which the Continental

hop is most suited and the English not popular. If even a small export trade could be built up it would provide a cushion very helpful to brewers of this country. So far there has been little success outside a few breweries within the British Empire.

DISCUSSION

The CHAIRMAN (Mr. B. Wood) said he was not worried about a shortage of seeds in the hop cone, but the question of P.V. was debatable; thus, he had kept samples of beer brewed with a very low hop-rate for six weeks, and found the beer excellent; in some country districts it was selling in fact at a greater age than six weeks. With regard to acreage and superfluous hops, he would suggest that present-day beer would not stand a high hop-rate, and that the public palate had been educated to such a beer.

Mr. H. J. Cox said that as brewers had been warned that they would probably be forced to take a further 1, 2, or 3 *per cent.* above the allotment of 88 *per cent.*, it would appear fair that no hops should be exported until every individual brewer had satisfied his requirements, as, otherwise, good hops might leave the country and a brewer needing more than his quota would have to choose from the inferior hops still available. With regard to planting up more Goldings, it was frequently said that soon there would be no such thing as a human picker and if all hops were to be machine-picked it would seem unwise to increase the Golding acreage. As to seedless hops, it was said by the older school of brewers that these were apt to give rise to filtration difficulties in the hopback. Although it had been claimed that lightly-hopped beers had stood up for six weeks, it should be remembered that in many public-house cellars this would not happen under the varying conditions and practices.

Mr. G. GASCOYNE asked whether it might be possible to correct the acreage position if the Hops Marketing Board represented to growers that it was in their own interest to adopt a policy of replanting each year 10 *per cent.* of their acreage. This would adjust the acreage to what was really needed, and on an average better hops would be produced from young stock. He asked further whether there was not some hope that English hops could be exported in place of the pre-war export of German hops; he understood that German hop-growing was likely almost to disappear. He also gathered that there were great difficulties in the way of exports from Czecho-Slovakia; he knew that business had been offered to Czecho-Slovakia which it had been impossible to put through.

Mr. J. W. SCOTT said that he understood the cold-storage position was unlikely to improve for a considerable time, and it was difficult and unsatisfactory for brewers to store hops on their own premises. He considered that the pocket was a bad container for storage purposes, taking up an excessive amount of room; why could not hops be baled? Oiled paper might be considered as an internal lining, allowing economy in view of the jute shortage by the use of a thinner outer cover.

Dr. R. H. ROBERTS, referring to the threat from *Verticillium* wilt, and the fact that hops were being

produced surplus to requirements, suggested that it would be advisable to lay the infected areas fallow instead of devoting so much expensive research to producing varieties—not altogether acceptable to the brewer—which could be grown in that acreage.

Mr. G. V. SMITH, deploring the delay in fixing hop prices, suggested that steps should be taken either to speed up the process or to give some early close indication to the trade of what the price was likely to be.

Mr. W. GLEW said that brewers realised that the jute position was difficult but, in spite of care in opening pockets, he was frequently disappointed at the low grading given to cloths by the Central Pockets Committee.

Dr. R. G. AULT said that it had been stated that the new varieties of hops lost P.V. much more quickly during storage than did the established English varieties; if this were so, it obviously detracted considerably from their brewing value. He would like to know on what foundation the statement was made, and whether the tests had been carried out on hops in cold store or ordinary store. With regard to Fuggles, the P.V. of which was generally somewhat low, he wondered whether any work had been undertaken with a view to improving the resin content of this hop.

Mr. M. CRAVEN SMITH considered that, despite the prominence given to them and favourable reports from some quarters, the new varieties were disappointing and not so suitable for brewing as first-class Goldings or Fuggles. He thought that growers might be disappointed if they expected big demands for new varieties in preference to Goldings and Fuggles.

Col. RICHARDSON, in his reply, said that the only way to fill an export order to-day was to re-purchase hops from a brewer; hence, the Hops Marketing Board's reserve was not affected in any way. If a brewer expected to be short of hops, he should get in touch with a merchant; at present, letters were being received from brewers saying that they had too many hops and asking if they could be relieved of them. He would be glad to find an outlet for them, not having been aware that redistribution of barrelage would cause a shortage of material.

He thought that the suggestion that growers should correct any present surplus in supplies by replanting 10 *per cent.* of their acreage was an excellent one, but he believed that in effect good growers already did this as part of their normal practice. The life of a hop garden was taken as 20 years, and after hop-picking he thought it would be found that a hill here and there was selected for re-planting; the good grower aimed to replace about 10 *per cent.* of his hills annually, and if that policy were generally followed it would cut down the surplus and also produce better stock for the future. Dr. Roberts' suggestion was also excellent, but it was difficult to persuade individual growers, who after experience of overcoming downy mildew thought the same would happen with *Verticillium* wilt. However, grubbing and fallowing appeared to be the only answer. As to wilt-resistant varieties, he would go no further than saying that they showed a tendency to be resistant; experiments must go on, but it might be well to try them on infected land.

He was not aware of any shortage of German hops; there was great difficulty last year in disposing

of hops from Bavaria. The difficulty in getting Czech hops was political, rather than one of production; he was informed that the crop had been sold quickly, but members would be surprised to know how many Czech hops came back on the market in April and June. This country's main difficulty in building up an export business was that a seedless hop with an aroma different from the English was preferred for lager brewing.

The putting of English hops into rectangular bales had been considered carefully, but the main difficulty was that with the present shortage of machinery for replacement it would be impossible to change the presses at present in use. Baling would also make it possible to pack in another material such as thick paper (if available); there would not be the same strain as in the pocket press. He would like to see rectangular bales, but it might not be easy to persuade growers, whilst it might be even more difficult to induce the Hops Marketing Board to countenance two kinds of package during the transition period. The question of encouraging Goldings had to be approached through the Board and the Hops Committee of the Brewers' Society, whereas in a free market it would be settled differently. At the present time they were subject to a formula for the issue of a basic quota, and there was no way of getting it altered without lengthy round-table arguments with the organizations concerned. He agreed that the delay in price fixing was unfortunate, but until more settled conditions returned and they had several years with the average price unchanged, he did not see how present difficulties could be overcome.

Machine-picking of Goldings was a serious problem, and judgment should probably be reserved until it was seen whether a machine could be produced which picked Goldings satisfactorily. It might well be necessary to pick Goldings by hand

while main-crop Fuggles were picked by machine. Like the combine harvester, the picking machine was coming to stay, and they would have to make it work. If it would not pick Goldings, it would have to be used with other hops. It was not a satisfactory solution of the problem, but the machines had to be used. He could inform the meeting that a careful examination of returned pocket-cloths was made, and if there were any complaints about the grading of pockets the Secretary of the Central Pockets Committee would take the matter up.

Most of the new varieties were introduced because of their higher P.V.; it was agreed, however, that the value was lost more quickly than in the case of established English hops. Work on the subject was now proceeding and would shortly be published. He himself had kept some samples of Brewers' Gold and Bullion in storage for two or three years. They did lose P.V., but more slowly in cold store than if not cold stored. He knew of no experiments to improve the P.V. of Fuggles which, apparently, merely varied with the growing conditions of the season. A recent Conference had brought out the fact that many brewers were not at all interested in new varieties. Main interest, restricted to a few brewers, was in Brewers' Gold and Bullion, which were substitutes for American hops. It was probably wrong to assume that P.V. was the over-riding character desired in a hop, for the vast majority of brewers were interested in aroma, which was imparted as flavour to the beer. However, there were some brewers, particularly of black beers, whose interest was mainly in the beer keeping as long as possible. It was often forgotten that the majority of breweries in the United Kingdom were of medium and small size, and could not use any of the new varieties even if they wished to do so.

COMMUNICATIONS

"WOODY" FLAVOURS IN DRAUGHT BEERS

By B. M. BROWN, B.Sc., F.R.I.C., E. C. BARTON-WRIGHT, D.Sc., F.R.I.C.,
and A. I. L. LYON

It has been found that flavours in draught beers described as "woody" or "musty" can be caused by species of *Actinomyces*. The organisms do not appear to grow in beer but in the wood of the cask while empty. Ordinary washing does not always remove the flavours.

AN occasional complaint about draught beer in cask is that it has a "woody" or, less frequently, a "musty" flavour. The present investigation into the cause of this flavour arose from the suggestion by one of us in 1947 that it might be due to *Actinomyces*. The *Actinomyces* form a large and important group of organisms found mainly in the soil. They are mostly saprophytes, *i.e.*, organisms

living on decaying vegetable or animal matter. They are difficult to classify with either fungi or bacteria as they have characteristics common to both groups; one of their distinguishing features is the possession of an extremely fine threadlike mycelium. In artificial culture the *Actinomyces* generally produce a characteristic unpleasant "earthy" smell.

In the early stages of this investigation repeated attempts were made to isolate *Actinomyces* from "woody" beer. Results were invariably negative. Microscopical examination of the beers or of the sediments after forcing the beers showed only the normal microbiological population of draught beers, and forcings seldom exhibited the "woody" flavour. Full cultural details for the *Actinomyces* were kindly supplied by the D.C.L. Laboratories at Epsom, where extensive work has been carried out on these organisms in connection with the production of antibiotics. It was then realized that beer was an unsuitable medium for their growth since their normal habitat is much more alkaline; in artificial media they grow best at a p_H of 6.8-7.0. It was unlikely therefore that they would be found growing in beer which normally has a p_H of 3.8-4.2.

Attention was then directed to the possibility that the organism was growing on the inner surface of the cask. Shavings were taken from four casks which had contained beer complained of as "woody," and were placed in a suitable medium for incubation. From three out of the four casks examined a growth of *Actinomyces* was obtained after about 12 days' incubation at 80° F. A further examination was made of seven other casks. Four of these were ordinary oak casks which had contained beer with a "woody" flavour and three were casks made from laminated wood which had contained beer with an abnormal flavour sometimes described as "woody" and sometimes as "resinous." In this case, growth of *Actinomyces* was obtained from the shavings from the ordinary casks but none from the laminated shavings. A search at the same time for possible sources of infection showed that *Actinomyces* were widely distributed in the cask-washing yard. They were also found on the platform and on the brushes of the cask-washing machine.

The next stage was to attempt to produce the offending flavour in casks which had been deliberately inoculated with cultures of *Actinomyces*. For this purpose isolates from the brewery were used together with two known strains from outside sources, including a specimen of *Streptomyces griseus*, a well known *Actinomyces* strongly resembling those isolated by us.

The experiments which are described in Tables I-IV show clearly that the "woody"

flavour can be communicated to the beer from a culture of *Actinomyces* growing in the empty cask.

TABLE I

PINS FRESHLY WASHED; INFECTED WITH 10 ML. OF LIQUID CULTURES OF THE ORGANISMS, BEER RACKED ON TOP AND FINED. AN UNTREATED CASK WAS USED AS A CONTROL.

Organism.	Flavour of beer	
	at 7 days.	at 12 days.
Control: No organism added	Normal	Normal
TC 48 (<i>Streptomyces griseus</i> from DCL)	Faintly woody	Woody
1047 <i>Actinomyces</i> sp., from a "woody" cask	Normal	Normal
" " " "	Normal	Normal

In this case no woodiness was obtained from the brewery isolates, but it was considered that not sufficient time had been allowed between infecting the cask and filling with beer.

TABLE II

PINS INFECTED AS BEFORE BUT FIVE DAYS ALLOWED TO ELAPSE BEFORE FILLING WITH BEER.

Organism.	Flavour at 7 days from rack.
Control: No organism added	Woody
TC 48	Woody
TC 51 <i>Actinomyces</i> sp. Meredith (National Type Collection No. 6833)	Woody
TC 52 from cask washing yard	Woody
G.1047 from "woody" cask	Woody
G.1047 " " " "	Woody

There was a strong development of the woody flavour in all casks, including unfortunately the control which presumably started or became adventitiously infected.

TABLE III

TWO CONTROL PINS WERE USED IN THIS EXPERIMENT AND THE TIME BETWEEN INFECTION AND FILLING WITH BEER WAS REDUCED TO 24 HOURS.

Organism.	Flavour at 11 days from rack.
Control	Normal
Control	Normal
TC 52	Slight "woody" flavour
G.1047	"Woody"

TABLE IV

IN THIS EXPERIMENT DIFFERENT ISOLATES WERE USED AND THE CONDITIONS KEPT THE SAME

Organism.	Flavour at:		
	2 days.	4 days.	6 days.
Control	Normal	Normal	Normal
G.1565. <i>Actinomyces</i> from "woody" cask from trade	Musty	"Woody"	Musty
G.1853. " "	Normal	Normal	Normal
G.1854. " "	"Woody"	"Woody"	"Woody"

In the results shown in Table IV, the control remained normal throughout; one of the infected casks failed to produce any abnormal flavour; one produced a musty taste and odour and one produced the typical "woody" flavour. No doubt the quality and intensity of flavour produced depends on the nature and vigour of the infecting organism.

Experiments were also carried out to determine if washing on a Goliath machine would remove the flavour. A number of casks from the above experiments were washed normally on the machine and allowed to stand empty for 24 hours before filling with beer. Table V shows the results.

TABLE V

Original infection:	Flavour after washing the cask 4 days after racking.
Control	Normal
TC 48	"Woody"
TC 51	Trace of "woodiness"
TC 52	Trace of "woodiness"
G.1047	"Woody"
G.1047	"Woody"
Control	Normal
Control	Normal
TC 52	Normal
G.1047	"Woody"

It is quite evident that ordinary washing fails to remove "woodiness."

Three of the above casks, *i.e.*, a control cask, one showing a trace of "woodiness," and one showing definite "woodiness," were again washed on the machine, allowed to stand 24 hours and again filled and tested (Table VI).

TABLE VI

Original infection.	Flavour of beer after first washing.	Flavour of beer after second washing.
Control ..	Normal	Normal
TC 52 ..	Trace of "woodiness"	Normal
G.1047 ..	"Woody"	Faintly "woody"

It will be seen that washing twice gives no guarantee of removal of "woodiness" although the flavour has improved.

GENERAL CONCLUSIONS

Off flavours in beer described as "woody" or "musty" can be produced as the result of access of strains of *Actinomyces* to the empty cask. Possible sources of infection have been shown to be the cask-washing yard and the brushes on the cask-washing machine. Washing a "woody" cask on the cask-washing machine does not remove the flavour.

The strains of *Actinomyces* isolated during this enquiry have been shown to be closely allied to *Streptomyces griseus*. This has been confirmed by the kind co-operation of workers in the D.C.L. Laboratories and in the laboratories of Messrs. Boots at Nottingham. Plates 1, 2 and 3 show the white growth on solid media of *Actinomyces* from shavings from "woody" casks and from swabs from the cask washing yard.

We have to thank the Directors of Whitbread & Co., Ltd., for permission to publish these results.

Chiswell Street,

London, E.C.1.

5th November, 1948.

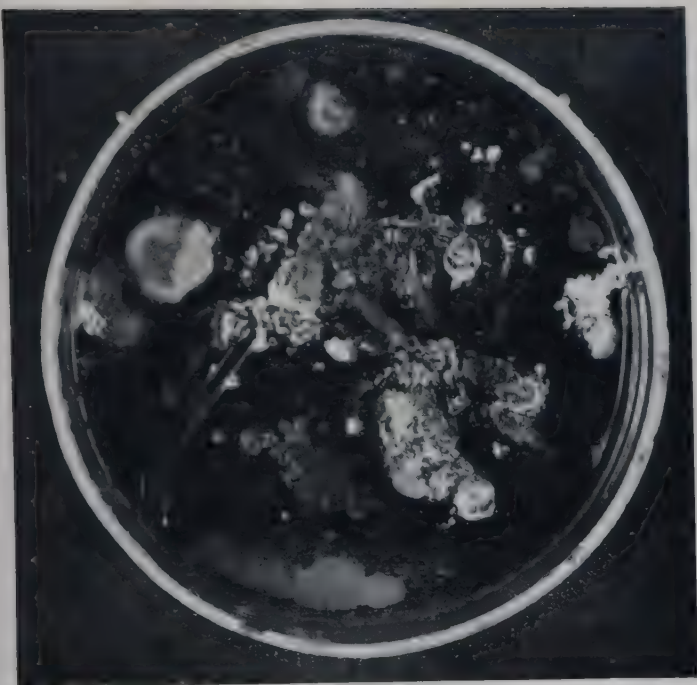


PLATE 1

Shavings from "Woody" Casks, showing growth of Actinomycetes (Sp.)

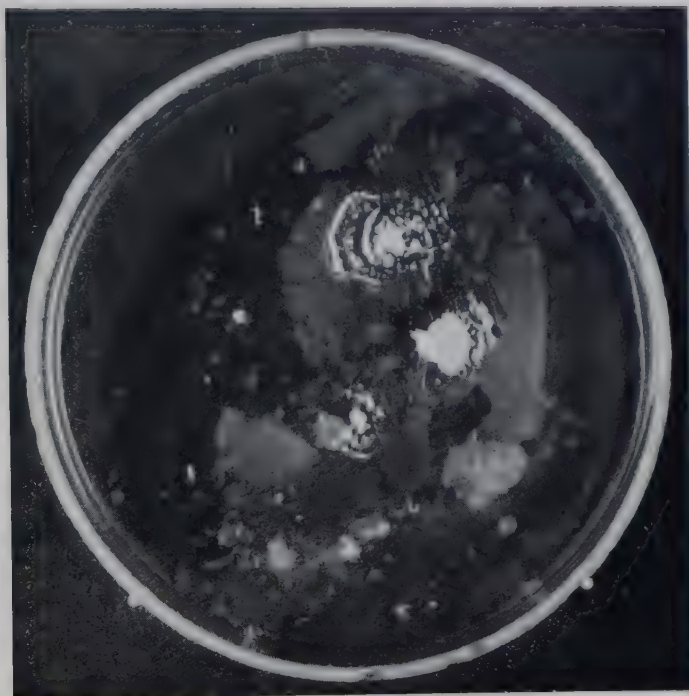


PLATE 2

Shavings from "Woody" Casks, showing growth of Actinomycetes (Sp.)

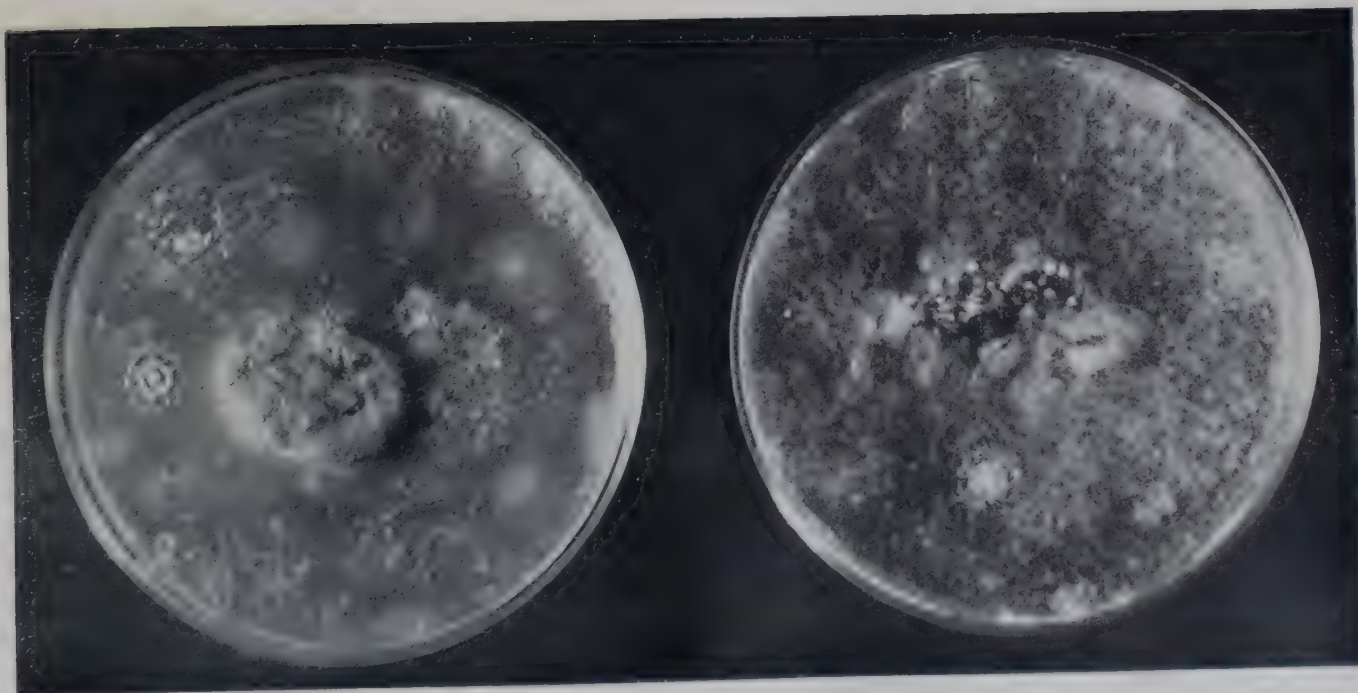


PLATE 3

Swabs taken from Cooperage Floor, showing White Colonies of Actinomycetes (Sp.)

JOINT ACTION OF α - AND β -AMYLASES. I. DETERMINATION OF REDUCING GROUPS AND OF AMYLOLYTIC ACTIVITY

By I. A. PREECE, M.Sc., Ph.D., F.R.I.C., and M. SHADAKSHARASWAMY, M.Sc., A.R.I.C.

The convenient ferricyanide method of reducing group determination has the disadvantage, when applied to starch hydrolysis products obtained with α -amylase, that it gives higher results than are obtained with copper methods; there is reason to suppose that the latter give the more correct results. Difficulty is thus introduced into the interpretation of amylase activity results when ferricyanide is used.

The relationship between results of reducing group determination by the two methods has now been established for a range of α -amylase conversion conditions, and it thus becomes possible for results obtained by one method to be expressed in terms of the other, whether as D.P. or α -activity in Lintner degrees or as maltose equivalent; expression as maltose equivalent (copper reduction) is to be preferred.

INTRODUCTION

A report is presented elsewhere of the application of a modification of the Hagedorn-Jensen ferricyanide method of sugar determination to a study of the activities of α - and β -malt amylases when acting individually or jointly on soluble starch substrates (Preece and Shadaksharaswamy, *Biochem. J.*, 1949, 44, 270). In this work, use was made of precipitated preparations of the two enzymes; no trace of β -amylase activity could be detected in the α -amylase from malt, whilst α -amylase contamination of the β -amylase preparation from barley, if indeed present, accounted for no more than 1/750 of the total activity of the preparation, a proportion which could have no significant influence in the types of conversion planned. Moreover, the preparations were free from maltase.

The results obtained are of some importance in connection with the assessment of amylolytic activity and may be briefly summarized, it being noted that the conversions involved the use as substrate of 1 per cent. or 2 per cent. soluble starch solution buffered to p_H 4.6, the conversion temperature being 21° C. (70° F.). Ferricyanide-reducing group production by β -amylase was linearly related to time up to 15 per cent. hydrolysis (calculated as apparent maltose per cent. of total theoretical maltose), whilst for joint action of α - and β -amylases hydrolysis/time linearity persisted to approximately 20–25 per cent. hydrolysis. With α -amylase, the relationship was less simple, and the contention of E. Kneen and R. M.

Sandstedt (*Cereal Chem.*, 1941, 18, 237) that for this enzyme there is no region of true linearity was supported. Nevertheless, so long as conversion was not unduly prolonged (not more, e.g., than 30 minutes), departure from linearity up to 10 per cent. hydrolysis was slight, and little error was, in fact, involved if linearity was assumed up to 15 per cent. Again, in joint action for a range of β : α activity ratios varying from 4 : 1 to 1 : 4, the actions of the two enzymes were truly additive up to 20–25 per cent. (i.e., to the limit of linearity of the joint action/time curve), the two enzymes appearing to act substantially independently within this range of hydrolysis.

Thus, taking these findings into account, it is possible to use the ferricyanide method for comparing the activities of two α -amylase preparations or of two β -amylase preparations. Similarly, the ferricyanide-reducing group producing potentialities of two mixtures of α - and β -amylases may be compared and, the activities of the α - alone being known in each case, the activities of the β -components can be calculated by difference. Superficially, the application of this to diastatic power determinations would appear obvious, for if the D.P. of a malt and its α -amylase activity are determined by the method of F. R. Graesser and P. J. Dax (*Wallerstein Lab. Commun.*, 1946, 9, 43) or of Preece (this *Journ.*, 1947, 154), β -amylase activity is obtainable by difference. There seems no reason to doubt the validity of the difference principle, but real difficulty does arise in the interpretation of the α -amylase results.

In this connection, an observation of first importance is recorded by J. Blom and C. O. Rosted (*Acta chem. scand.*, 1947, 1, 32; this *Journ.*, 1948, 111), who note that a mixture of mono-, oligo-, and polysaccharides such as might result from the α -amylase conversion of starch gave hydrolysis corresponding to a production of 34.3–34.9 *per cent.* of theoretical maltose as determined by copper reagents (*e.g.*, Fehling's solution), but 42.0 *per cent.* as determined by ferricyanide. Thus, the ferricyanide technique gives enhanced values for reducing power as compared with Fehling's solution due partly, these authors suggest, to a partial oxidative rupture of certain linkages in the higher saccharides. Agreement may be expressed with these authors that, whilst copper reagents do not necessarily give the true absolute values for reducing group production (*i.e.*, they are not themselves necessarily entirely free from this oxidative-disruptive tendency), they may be assumed to give the more nearly correct results. Accordingly, it is proposed to regard copper methods as standard, a proposal which is further strengthened by the past wide-spread use of Fehling's solution in D.P. determination, and the fact that the traditional "Lintner degree" is based on the use of this reagent. Also, as will appear, there is no reason to suppose that the Fehling's and ferricyanide methods give other than concordant results in β -amylase conversions, providing thus a common basis for calculation.

It may be asked why, in view of this difficulty, it is considered worth while to investigate further the use of ferricyanide in amylase activity (including D.P.) determinations. There are several reasons. Thus, the end point of Fehling's titrations is not always sharp even when using methylene blue as indicator as described in *Standard Methods of Analysis* (this *Journ.*, 1948, 179), and this is particularly the case when α -amylase conversions are being examined. However, this objection carries little weight with the experienced worker and might be obviated altogether by adoption of some such modified procedure as that recently proposed by Blom and Rosted (*Acta chem. scand.*, 1947, 1, 382; this *Journ.*, 1948, 165). More important is the fact that the Fehling's method cannot be easily directly applied to such dilute solutions (in respect of reducing groups) as are encountered with controls when carrying out

other than routine experimental work with amylases. The iodimetric method, frequently employed for such purposes, may give somewhat higher results than Fehling's solution in presence of higher saccharides, and may also on occasion give end-point difficulties.

On the other hand, the ferricyanide technique is being increasingly employed in investigations on amylolysis. It is simple to carry out, though it can scarcely be said to save appreciable time as compared with the older method; it can, however, be used for even dilute control solutions without any difficulty. Also, the end-point is reasonably sharp. Accordingly, it becomes of interest to investigate the relationship of results obtained by Fehling's and ferricyanide titrations, with special reference to the determination of amylolytic activity, and to establish the conditions which should be observed when carrying out amylase determinations by either method.

EXPERIMENTAL

Determination of Reducing Groups.—Two principal methods have been used. The ferricyanide technique has been described elsewhere (Preece and Shadaksharaswamy, *loc. cit.*), whilst the Fehling's method is that of the *Standard Methods* (*loc. cit.*). On occasion, use has also been made of the method of Bertrand under the conditions described by C. A. Browne and F. W. Zerban (*Sugar Analysis*, 3rd ed., John Wiley & Sons, New York, 1941, page 801). The methods have all been standardized against the same sample of maltose, giving accordingly over the ranges employed concordant results for this sugar. Such standardization is quite essential, in view of discrepancies which may arise in a given method from slight variations of technique. The ferricyanide procedure is, perhaps, particularly susceptible to such variation (see, *e.g.*, L. H. Lampitt, C. H. F. Fuller, N. Goldenberg and G. H. Green, *J. Soc. chem. Ind.*, 1947, 66, 68).

Enzyme Sources.—For some conversions, preparations of precipitated α - and β -enzymes were used, singly or together in different proportions, these having been obtained by methods already outlined (Preece and Shadaksharaswamy, *loc. cit.*). In other cases, the standard 5 *per cent.* extract of malt was prepared as used in D.P. determinations

(this *Journ.*, 1948, 179), this being used either directly as a source of both amylases, or after inactivation of β -amylase by maintenance at 70° C. (158° F.) for 15 minutes in presence of 0.2 grm. of calcium acetate *per* 100 ml. of extract.

Soluble Starch Substrate.—Where malt extract was used for conversion, the substrate employed was 2 *per cent.* (air-dry) soluble starch solution buffered to p_H 4.6 (acetate buffer). This concentration corresponds to 1.753 grm. dry starch *per* 100 ml. With precipitated enzymes, the substrate was soluble starch solution normally buffered at p_H 4.6 and containing, after addition of the enzyme solution, 1 grm. of dry starch *per* 100 ml. of conversion liquor; in two cases, a p_H of 5.4 was used. Results with both substrates, and therefore with precipitated enzymes and malt extracts, and also at both p_H values, are not inconsistent with the view that the varied methods of working do not substantially affect the results obtained (with a possible exception discussed below), and individual results are accordingly collected in the summarized Table II without distinction of method. Similarly, the Table contains results obtained both by Fehling's and Bertrand's method of sugar determination, these again showing inappreciable deviations.

Conversions.—These were carried out uniformly at 21° C. (70° F.) for varying periods. Reaction was then stopped by adjustment of p_H to 10.5–11, sugar determination following after appropriate dilution. It will be observed that the 60-minute conversions using malt extract followed closely the technique of D.P. determination.

Relationship of Ferricyanide to Fehling's Results.—In the case of α -amylase conversions, a factor (f) has been calculated directly from the ratio of reducing group production assessed by ferricyanide to reducing group production assessed by Fehling's titration (or, in a few cases, by Bertrand's method); alternatively, for purposes of report, it is more convenient to express f as the ratio of *per cent.* reducing group production (apparent maltose on dry starch) by the two methods. In the case of joint action of α - and β -amylases, f is calculated indirectly from the relation:

$$\beta + f\alpha = f'(\beta + \alpha),$$

where β and α are the concentrations of the two enzymes (degrees Lintner as determined by Fehling's titration) and f' is the observed ratio for the joint action. Examination of β -amylase conversions shows that results by the two methods of sugar determination do not there significantly differ. Thus, 10 determinations of the ratio ferricyanide/copper-reducing groups (as maltose) in β -amylase conversions ranging from 16.1 *per cent.* to the end-point of hydrolysis gave results in the range 0.99–1.03, with a mean of 1.01 ± 0.01 ; this does not differ significantly from unity.

Results.—It is unnecessary to give in detail the whole of the results obtained. However, in Table I and Fig. 1 are shown the results of two series of starch conversions carried out with extracts from two different malts, β -amylase having been inactivated by heat treatment. The higher conversion values in each case obtained by the ferricyanide method are clearly shown. Moreover, it is apparent that, for copper assessment (and has already been shown for the ferricyanide method), little error is incurred if linearity with time of hydrolysis is assumed up to 15 *per cent.* hydrolysis.

This being so, a value for f in each series can be obtained for the 0–15 *per cent.* phase of hydrolysis from the ratio of the initial slopes of the appropriate curves; for each series, the value so obtained approximates closely to 1.25. Beyond the 15 *per cent.* stage, the ratio diminishes progressively.

In Table II are summarized the results of 50 determinations of the value of f under a variety of conditions. These results are presented in three series. In series (i) are included values obtained indirectly from the results of joint action of the two amylases, the values given for *per cent.* conversion being the increments due to α alone. In series (ii), a similar range of conversions is included (up to approximately 12 *per cent.*) for α -amylase acting alone. No distinct trend in the value of f is seen in either series, and it may be assumed in this early stage of hydrolysis that it is substantially constant, approaching closely the figure 1.25. It would of necessity follow that the value must be constant if the reaction were truly linear during this phase. In series (iii) the position is different, with the results for f showing a definite downward trend with increasing hydrolysis. The inverse correlation between

f and R_{Cu} can be expressed in the form of the equation shown in the Table; whilst it should not be inferred that a strict linear relationship does in fact exist, this equation allows calculation of figures which closely accord with those actually observed. The 50 results of Table II are shown graphically in Fig. 2, the scatter in which indicates the degree of precision obtainable in such measurements.

W. N. Haworth, A. Macey and S. Peat, *J. chem. Soc.*, 1948, 924; this *Journ.*, 1948, 340). Myrbäck (*loc. cit.*) further claims that the initial action involves the liberation of glucose. Although for a particular starch unit the reactions must necessarily follow in the order given, it appears unlikely (Preece, *Chem. & Ind.*, 1948, 611) that separately consecutive actions are to be found throughout

TABLE I
HYDROLYSIS OF SOLUBLE STARCH BY α -AMYLASE
(as followed by ferricyanide and by Fehling's titrations)

Duration of conversion (hours).	Reducing group production as apparent maltose per cent. of dry starch.				Value of $f = \frac{x}{y}$	
	Series A.		Series B.		Series A.	Series B.
	Ferri-cyanide method = x .	Fehling's method = y .	Ferri-cyanide method = x .	Fehling's method = y .		
0.5	10.35	8.58	6.90	5.43	1.21	1.27
1	18.86	15.82	12.41	9.94	1.19	1.25
1.5	26.39	21.71	17.63	14.61	1.22	1.21
2	—	—	23.01	18.65	—	1.23
2.5	—	—	27.48	23.83	—	1.15
3	38.07	32.89	30.79	26.92	1.16	1.14
4.5	41.53	36.95	—	—	1.12	—
5	—	—	39.85	35.60	—	1.12
24	51.89	50.16	51.68	48.90	1.04	1.06

DISCUSSION

It should be noted that conversion figures given in this paper are expressed in the form: apparent maltose per cent. of dry starch; if it is preferred to think in terms of theoretical maltose production, the figures given should be divided by 1.055.

The results of Table II indicate that the degree of precision obtainable in determinations of f is not high. This is perhaps not surprising when it is noted that the amylase preparations and extracts used had their origin in a number of different malts. There appears every reason to suppose (see, e.g., K. Myrbäck, *Advances in Carbohydrate Chemistry*, 1948, 3, 251; R. H. Hopkins, *Advances in Enzymology*, 1946, 6, 389) that α -amylase action takes place in two stages—a rapid initial dextrinizing stage and a subsequent further slow hydrolysis of the initial dextrins with reduction of their molecular complexity, though without breaking the original cross linkages between chains in the amylopectin molecule (see also E. J. Bourne,

a particular starch conversion. Rather, a degree of overlapping is to be assumed between the rapid dextrinization of the starch and the slow saccharification of the dextrins. The influence of concomitants on the behaviour of α -amylase is well recognized (E. Kneen, R. M. Sandstedt and C. M. Hollenbeck, *Cereal Chem.*, 1943, 20, 399; this *Journ.*, 1943, 307; Preece, this *Journ.*, 1948, 141), and if it is conceded that concomitants and starch concentration (Michaelis principle) influence differently the two stages of the reaction, it would not be expected that two α -amylase preparations or extracts from different sources would give identical lines of reaction. Further, the degree of overlap varies with different concentrations of the enzyme, causing a raising of the level of the apparent inflection—suggesting a disproportionate increase in one phase—as the enzyme concentration is increased. Fuller evidence on this point will be presented in a report now in preparation. Thus, with different reactions

proceeding at different rates according to the conditions of experiment, it appears inevitable that values for f will vary between rather wide limits.

from the regression equation of Table II; the extrapolated values for 0, 5, and 10 *per cent.* conversion are not inconsistent with those experimentally determined in the

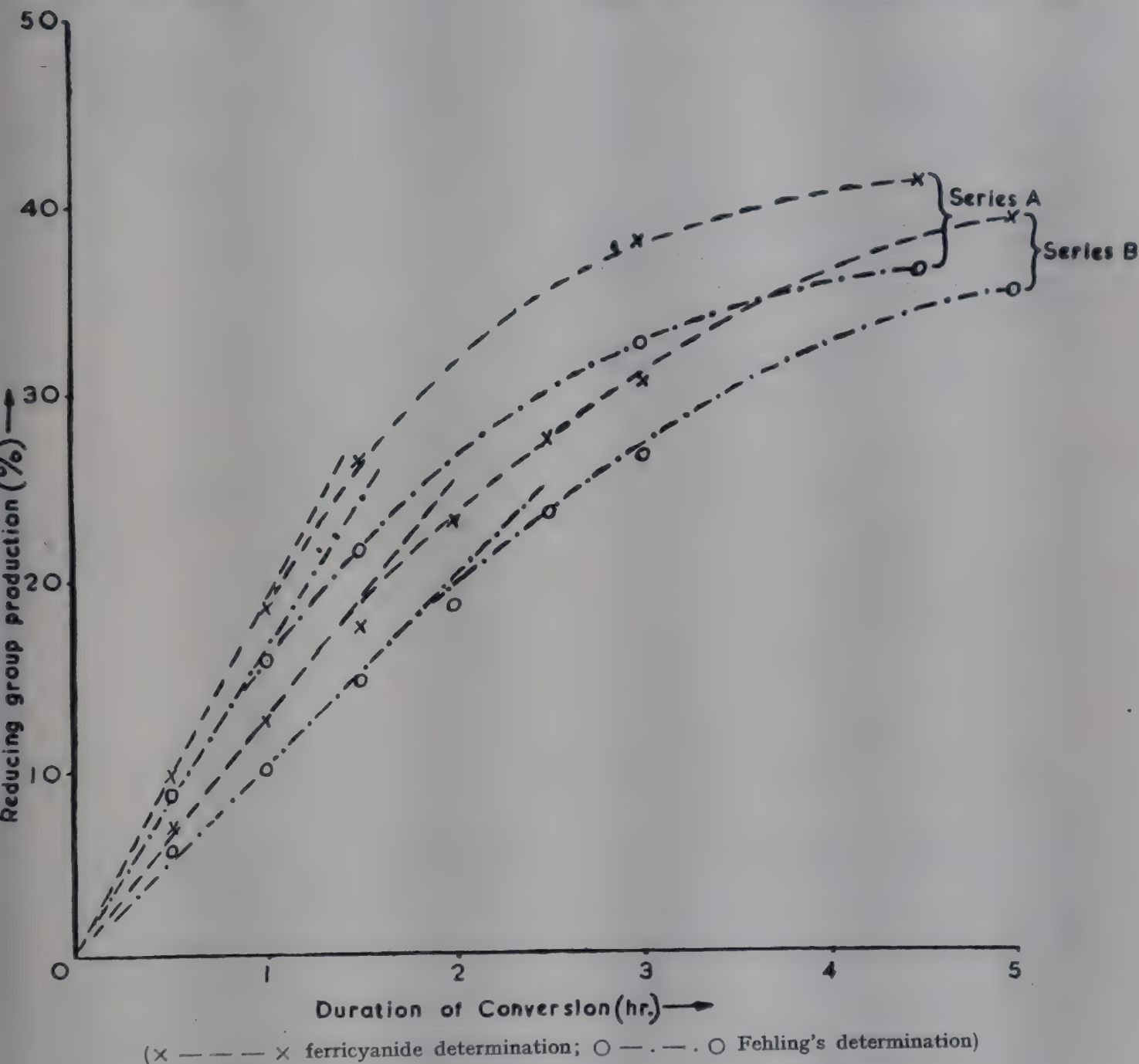


FIG. 1—Liberation of reducing groups from starch by α -amylase.

Recognizing these limitations of precision, it still appears that values for this factor of utility in routine work can be established from the results of Table II. For conversions in the range 0–12 *per cent.* (copper value), the mean factor 1.25 may be used. Mean values for the range 15–50 *per cent.* are shown in Table III, having been calculated

initial stages. Table III shows also the mean *per cent.* of “special reducing groups” (*i.e.*, those reducing ferricyanide but not, *e.g.*, Fehling’s solution) at each level. It will be observed that whilst the relative proportion of “special reducing groups” is greatest in the initial stages and falls thereafter, the absolute amount of such groups rises to a

TABLE II

VALUES OF THE RATIO (f) OF FERRICYANIDE/FEHLING'S (OR BERTRAND'S) REDUCING GROUPS AT DIFFERENT LEVELS OF HYDROLYSIS

(Figures quoted for *per cent.* hydrolysis (R_{Cu}) are those determined by copper reduction)

	Series (i) α in presence of β .	Series (ii) α alone.	Series (iii) α alone.
Range of R_{Cu} included	3.7 -12.1	5.3 -11.7	14.6 -50.3
Range of f observed	1.20- 1.31	1.20- 1.30	1.21 - 1.04
No. of results	8	16	26
Trend of f	None observed	None observed	Downward with increasing R_{Cu}
Mean value of f	1.241	1.253	—
Standard deviation of individual result	± 0.040	± 0.024	—
Standard deviation of mean	± 0.014	± 0.006	—
Correlation coefficient	—	—	-0.880
Regression of f on R_{Cu}	—	—	$f = 1.292 - 0.00436 R_{Cu}$ ± 0.027

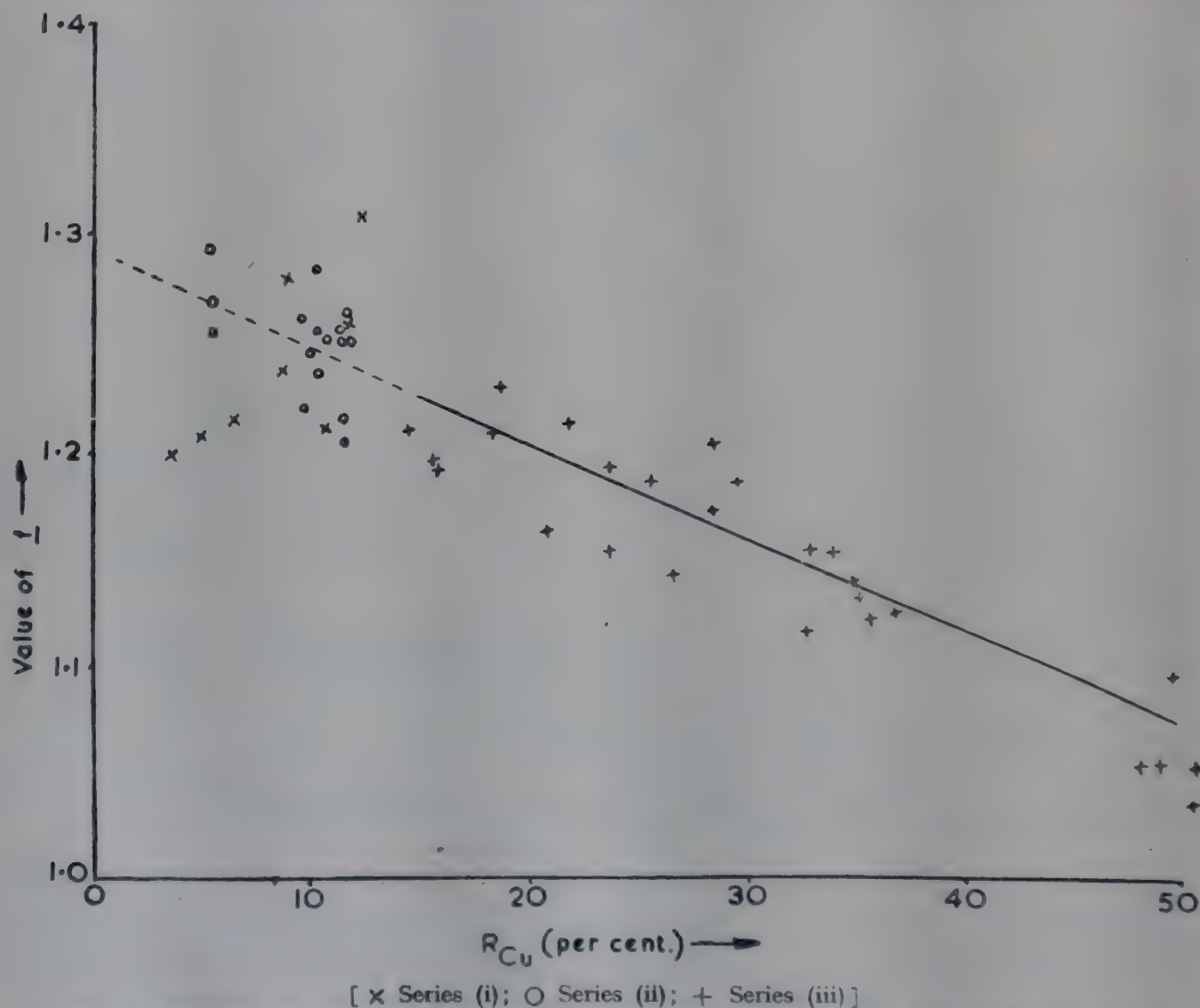


FIG. 2—Variation of the ratio (f) of ferricyanide/copper reducing groups at different levels of hydrolysis. The hydrolysis figures shown (R_{Cu}) are those for copper reduction. The calculated regression line (Table II) is drawn in.

maximum at 30–35 *per cent.* hydrolysis, and then falls. It is not proposed to discuss theoretical aspects of this finding at this stage, except to note that the values shown would conform to the suggestion that, in the earliest stages of hydrolysis, one special reducing group is “liberated” for each three “normal” (*i.e.*, copper-) reducing groups. However, there are obvious difficulties of interpretation, such as those due, *e.g.*, to the fact that the *f* value for glucose itself, a proportion of which may be formed at this stage, is of the order of 0.84–0.81 for concentrations of 0.1–0.2 *gram. per 100 ml.*

basis, and taking into account the conditions of the A.S.B.C. method, the divisors to convert ferricyanide maltose equivalent to degrees Lintner would be 3.85 for β -amylase conversions or 4.80 for α -amylase conversions; for a malt having a β : α -amylase ratio of 4 : 1 (Preece, this *Journ.*, 1948, 141), the factor would be 4.04. It should be noted that the value for malts will vary with the β : α ratio; the value of 4, mentioned above, is correct for a ratio of 5.33 : 1. Since it is impracticable to use variable factors for different malts, we propose to use the values 3.85, 4.05, and 4.80 for β , $\beta + \alpha$, and α

TABLE III
CALCULATED VALUES OF THE RATIO (*f*) OF FERRICYANIDE/COPPER REDUCING GROUPS
AT DIFFERENT LEVELS OF CONVERSION BY α -AMYLASE

Reducing groups <i>per cent.</i> of dry starch.			Value of <i>f</i> .
Copper method.	Ferricyanide method.	“Special groups.”	
0*	0	0	1.292
5*	6.35	1.35	1.270
10*	12.48	2.48	1.248
15	18.40	3.40	1.227
20	24.10	4.10	1.205
25	29.57	4.57	1.183
30	34.83	4.83	1.161
35	39.86	4.86	1.139
40	44.72	4.72	1.118
45	49.32	4.32	1.096
50	53.70	3.70	1.074

* Extrapolated values.

Amylase Activity Determinations.—Applying these findings to the use of the ferricyanide technique for the determination of diastatic power and α -amylase activity, it may be recalled that the American Society of Brewing Chemists (*Methods of Analysis*, Malt-6, 4th ed., 1944) obtain a value for D.P. by dividing the maltose equivalent of standardized conversions by the factor 4. Maltose equivalent represents the reducing groups liberated (calculated as maltose, and determined either by Fehling’s titration or ferricyanide) in 30-minute conversion of 2 *per cent.* buffered soluble starch solution brought about at 20° C. (68° F.) by 2 *ml.* of a 5 *per cent.* infusion of malt. In the standardization of the sugar determination methods used in the present work, it has been established that, for a 25 *ml.* titration, 5 *ml.* of Fehling’s solution are equivalent to 38.5 *mgm.* of anhydrous maltose. On this

conversions respectively, when maltose equivalent is determined by ferricyanide. There is no justification for using the factors 4.05 and 4.80 when Fehling’s solution is used; in this case, 3.85 is the appropriate figure to use with all three types of conversion. It has already been noted that the limits of substantial linearity for $\alpha + \beta$ and α -amylase conversions determined by ferricyanide are 20–25 *per cent.* and 10–15 *per cent.* respectively, and these limits should be observed when using the A.S.B.C. method (*loc. cit.*). For the Institute of Brewing method (*loc. cit.*), linearity to 30–40 *per cent.* conversion (“Kjeldahl’s law”) may be assumed for $\alpha + \beta$ conversions, but for α -amylase determination (Preece, 1947, *loc. cit.*) it is recommended that sufficient extract should be taken to give a conversion of not greater than 12 *per cent.*; expressed otherwise, the final Fehling’s titration for α -conversions

should be not less than 25 ml. Observing these precautions and using the appropriate conversion factors where necessary, results for D.P. and α should be obtainable by the ferricyanide method, and for α by Fehling's titration, with an accuracy comparable with that obtained in the traditional D.P. method, with its inherent shortcomings.

Protracted Conversions and those at Higher Temperature.—For α -amylase conversions at 21° C. (70° F.) carried to the 15–50 *per cent.* hydrolysis stage, it can be shown from the regression equation of Table II that the approximate relation between ferricyanide reducing value (R_{Fe}) and that obtained with copper reagents (R_{Cu}) is given by: $R_{Cu} = 148.1 - \sqrt{21933.6 - 229.4R_{Fe}}$, whence a value for copper reduction can be calculated when the more convenient ferricyanide method is used for determination. More simply, the figures in the first and second columns of Table III can be plotted against each other, interconversions then being read from the curve. It may be questioned whether it is allowable to use this relation when conversion is carried out at higher temperature. Temperature change, as has already been suggested for variations in concomitants, might be expected to influence the dextrinizing and saccharifying aspects of

α -amylase action to different extents, and so cause changes in the value of f . Further information will be presented on this point at a later date; in the meantime, the relationship expressed in this equation may be regarded as a useful first approximation for such conversions.

SUMMARY

1. In starch conversions made in presence of α -amylase, reducing group determination by a modification of the Hagedorn-Jensen ferricyanide method gives higher results than those obtained by the use of Fehling's solution.

2. For α -amylase conversions in the range 0–12 *per cent.* hydrolysis, the ratio of ferricyanide/copper-reducing groups (as maltose) approximates to 1.25; from 15–50 *per cent.* hydrolysis, this ratio falls according to the relation $f = 1.292 - 0.00436R_{Cu}$.

3. The implications of these findings in their relationship to D.P. and α determination by ferricyanide and by Fehling's solution are discussed.

Heriot-Watt College,
Edinburgh.
October, 1948.

VOLUMENOMETER METHODS I. DENSITY OF BARLEY AND MALT

By G. HAGUES and J. RUSSELL

THE work outlined below was carried out in an attempt to discover some criterion of the quality of barley and malt, and in particular to provide a sidelight on the quality of some Yorkshire barleys and malts on which brewing trials have been carried out in this brewery for the N.I.A.B.

It is comparatively easy to differentiate between the extremes of quality in barley and malt. E. S. Beaven, in his recent book (*Barley*, Duckworth, 1947), says:—

"The difference between steely and mellow grain can be judged from its appearance by all who are familiar with malting barley, and can be further demonstrated in several ways, most easily by the well-known 'Kornprüfer' or cutting instrument; also by the difference in translucency of the grains, when seen by ordinary transmitted light. Steely grain is translucent, probably because of the continuity of the cell contents: mellow grain is opaque. . . . Whilst the 'radiograph' of the

endosperm of steely grains appears quite uniform and structureless, that of mellow grains shows more or less numerous clefts or striations, as if the endosperm were split or discontinuous, and in malt the striations are so much more numerous as to amount to a network. It is probable that these are real air-spaces."

Perhaps the most interesting and illuminating contribution to our knowledge of the subject dates back to the publications of H. T. Brown (*Trans. Guinness Research Lab.*, 1903, 1, pages as cited) in which he discusses the physical characteristics of the grain, its "mealiness" and the probability of occurrence of air or vacuous spaces in the endosperm, and endeavours to correlate quality with this property and with density. In the first report, Brown says (*id.*, p. 109):—

"The proposal to determine the air-space in the grain from its specific gravity was first made by

E. S. Beaven, with a view to obtaining a further measure of the degree of 'mellowness' independent of the 'Kornprüfer' test. Unfortunately, it is impossible to draw a distinction between the air in the interspaces of the endosperm and that which is entangled in the tissue of the pales, and between the pales and the true envelopes of the caryopsis."

He then describes a method of density determination, using dry toluene to displace the air around the grain, and goes on to say (p. 120):—

"The interspaces of the grain which are determined by this method are those of the endosperm as well as the air-spaces in the tissue of the pales and those lying between the pales and the pericarp. From the practical point of view, it is the interspaces of the endosperm which are by far the most important, since they determine to a large extent the relative degree of 'mealiness' or mellowness of the grain.

"We can obtain a very fair idea of the relative distribution of the interspaces in the endosperm and integuments by comparing the distal halves of mealy and steely grains.

"In the completely 'steely' halves, the whole of the entangled air must be due to the envelopes of the grain, since the endosperms are free from interspaces, so that providing the extent of adhesion of the pales in the two cases is similar, the difference in air-content between the 'mealy' and 'steely' halves will be a measure of the air-spaces in the endosperms of the mealy halves."

At first, Brown thought that air might enter the corn at some point along the line of the furrow, but later, after using a skinless barley, came to the conclusion that the spaces in the grain were vacuous.

It seemed probable that the old and well-known volumenometer method might be applied as a way in which the density of malt or barley could be determined easily and simply without the contact of solvents and one which would be independent of the air under the pales. The apparatus as shown in the diagram (Fig. 1) was therefore set up, a flask of the ordinary distillation type, fitted with a side tube with a T-piece tap and a well ground glass stopper, being connected with a gas measuring tube and mercury levelling apparatus. All the essential parts were carefully calibrated, and the apparatus was used in the following way.

A weighed quantity of barley or malt was introduced into the flask until it was filled to the level of the side tube. With the tap open to the atmosphere, the glass stopper, well greased, was put in and weighted down with a piece of lead, which served to keep the stopper tight during conditions of

pressure in the flask, and also acted as a safety valve against its bursting. With the mercury level at zero, the tap was closed and the pressures necessary to bring the mercury to various levels in the measuring tube determined. The volume of air in the apparatus can be calculated from these figures and hence, by subtraction from the total volume of the apparatus, the volume of the

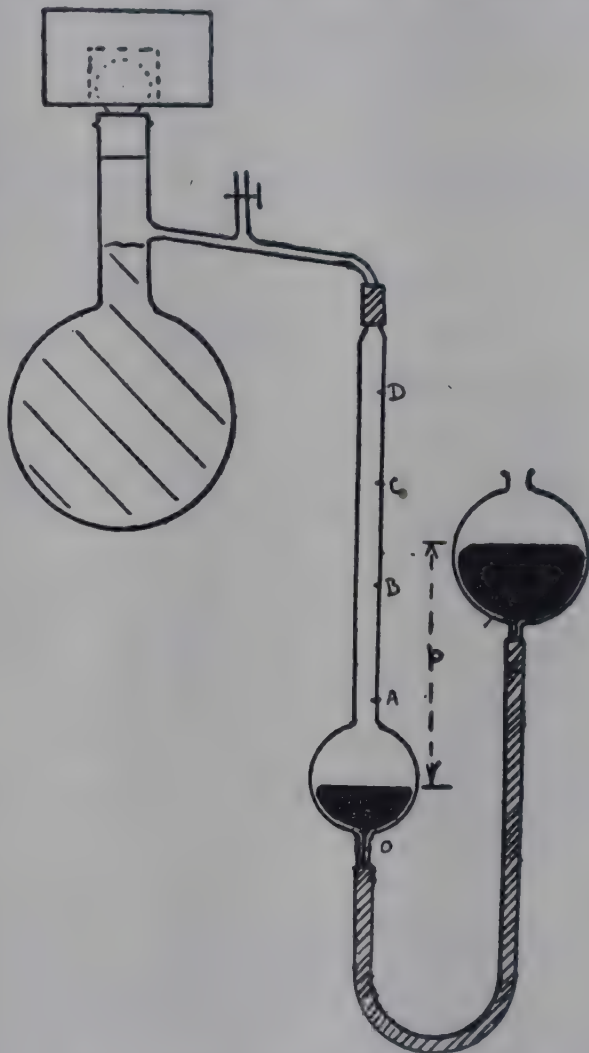


FIG. 1—The Simple Volumenometer

barley or malt can be found. Calculation of the density is as follows:—Let A = atmospheric pressure, p = manometric pressure applied, V = unknown volume of air in apparatus, and v = decrease in the volume of the air on applying the pressure p . Then

$$AV = (A + p)(V - v);$$

$$\text{i.e.,} \quad V = v(A + p)/p.$$

There are certain details to which attention must be given when setting up the apparatus. The measurements are most accurate when

the pressure and volume differences (both measured linearly) are of the same magnitude. Using this simple apparatus, an accuracy greater than three significant figures in the density of barley or malt is not claimed. The mercury was brought, in turn in every case, to the same four points A, B, C and D on the measuring tube and the level in the other limb of the manometer read by means of a vertical scale. The four density results thus obtained showed a maximum range of error of ± 0.01 , but were mostly identical.

The densities of a variety of barleys and malts were found in both the ground and unground states. In the unground state, a figure is obtained which embodies the solid material of the grain together with the gaseous or vacuous spaces, whichever they may be, which do not communicate directly with the exterior of the corn. The density of the ground material is its density without those spaces and is therefore greater, and the amount of air space can be calculated from the two figures.

TABLE I
DENSITY OF SAMPLES OF UNGROUND AND GROUND BARLEY AND MALT

Grain.	Dried barley.			Malt.		
	Density.		Spaces per cent.	Density		Spaces per cent.
	Unground.	Ground.		Unground.	Ground.	
<i>Experimental Barley—</i>						
Plot 1	1.37	1.46	6.2	1.35	1.53	11.8
Plot 2	1.37	1.46	6.2	1.16	1.46	20.6
Plot 3	1.37	1.45	5.5	1.32	1.62	12.3
Plot 4	1.38	1.43	3.5	1.32	1.53	13.7
<i>Normal Brewery Samples—</i>						
Hunts. 1	1.42	1.45	2.1	1.27	1.46	13.0
Hunts. 2	1.40	1.45	3.6	1.31	1.49	14.5
Yorks.	1.41	1.47	4.1	1.26	1.49	15.4
Green barley	1.42	1.42	nil			
Badly modified malt ..				1.25	1.47	15.0
Well preserved 1937 Californian malt ..				1.28	1.47	12.9

We were unable to identify a saturated vapour phase or adsorption effect, either of which would have vitiated our treatment of air as a perfect gas. The flask and measuring tube were calibrated in the usual way by filling with water and weighing, and the correctness of this was checked by setting up the apparatus, introducing weighed amounts of mercury into the flask and determining its volume volumetrically. Without taking stringent temperature precautions, the accuracy was quite sufficient to demonstrate the considerable differences which can exist in barley and in malt, and the method provides a simple determination which is easily carried out.

The existence of spaces was amply confirmed by our experiments, which indicated only a small volume of space in barley, but a large amount in malt up to as much as 20 *per cent.* of its volume. It had been anticipated that in a good, well-made malt, the density of the solid matter would be low and the "spaces" content high, whereas those of a badly modified malt would resemble more nearly those of a barley, but this was not so. Nevertheless, to the "bite" test, Plot 2 malt (see Table I) was very well modified, light and mealy, and in the brewing trials yielded the best extract in the brewery. It is also of interest to note that the results for a green barley indicate an absence of spaces.

VOLUMENOMETER METHODS

II. VOLUMETRIC CARBONATION OF BEER

By G. HAGUES

Carbonation is a most important process in the production of modern bottled beer and several methods for carrying it out are well known. The process described below, however, has not, to the author's knowledge, previously been outlined in the literature, though it formed the subject of a paper (unpublished) read to the London Section of the Incorporated Brewers' Guild on 25th January, 1937. Carbonation is a simple process, requiring a top pressure of carbon dioxide gas and a sufficiently large gas-liquid interface to encourage speed of solution, but

The method described below, which we have called "The volumetric carbonation of beer," makes it possible to use such simple, easily cleaned plant, and can be divided into two distinct processes: firstly, the measurement of the amount of beer in the tank to be carbonated; and secondly, the measurement of the gas to be dissolved.

The layout of the plant is given in the diagram. *A*, *B* and *C* are carbonating tanks, any one of which contains an unknown quantity of beer to be carbonated. *M* is a carbon dioxide gas-measuring tank. *G* is a

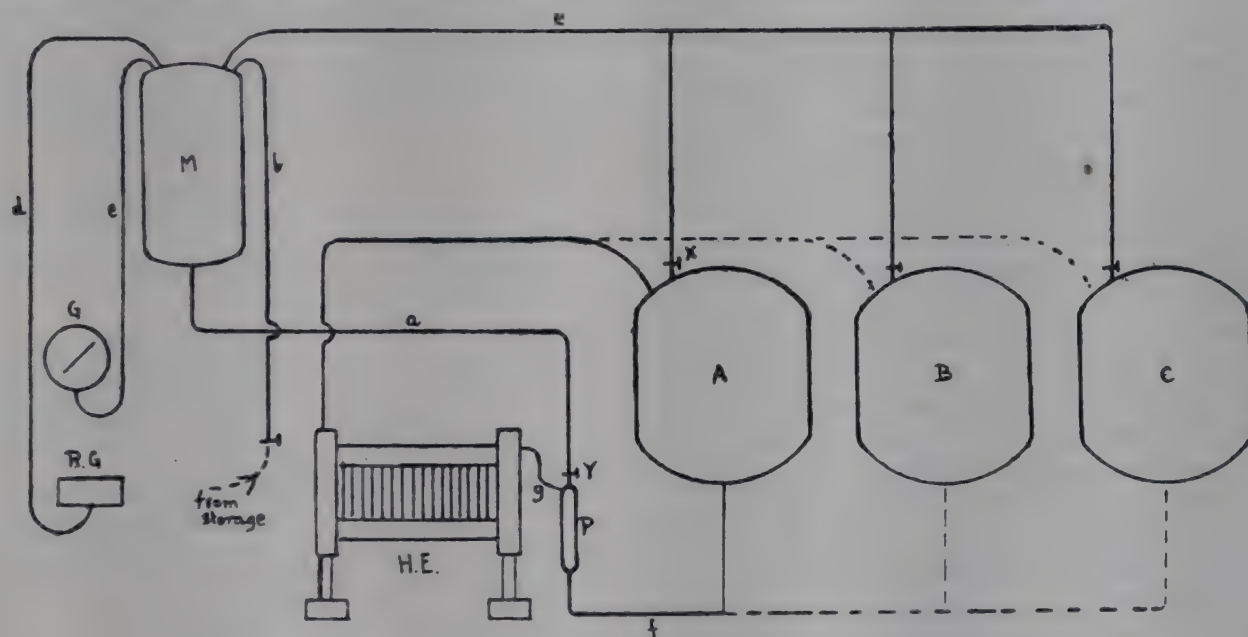


FIG. 1—Layout of Plant for the Volumetric Carbonation of Beer

exact carbonation, *i.e.*, the introduction of a predetermined measured quantity of gas *per* unit volume of beer is not easy without the use of apparatus which sacrifices some of its simplicity.

The modern trend of brewing plant design is to eliminate all pockets and crevices in which bacteria may thrive. Plant is now constructed of chemically resistant hard materials which are easily cleaned. The tank with sharp right-angled corners has made way for the vessel with rounded continuous surface and, wherever possible, fixed impedimenta and fittings such as sight glasses have been removed entirely.

large-faced, accurate pressure gauge and *R.G.* a recording pressure gauge. *H.E.* is a counter current heat-exchanger and *P* a centrifugal pump with a pipe attachment for introducing the gas. The pipes, represented by single lines and cocks, are shown in the appropriate places. The tanks *A*, *B* and *C* are as simple as possible. The beer return pipe from the circulatory system ends below the surface of the beer.

When it is necessary to carbonate beer in tank *A*, the air above the beer is first displaced by carbon dioxide gas until a test shows a purity of at least 99.5 *per cent.* The vessels *A*, *B* and *C* are cut off from the

gas measuring tank M by the taps shown at the top of the tanks, and tap Y shown on the pump is also closed. Circulation through the heat exchanger is started in order to chill down the beer. Gas from a storage tank is introduced into tank M until the gauge G shows some pre-arranged, arbitrary figure (30 *lb. per sq. in.* is convenient). The gas spaces over the beer in tank A and tank M are now connected by opening tap X and the gas pressures in the two allowed to equalize. When this is complete, the final pressure is read from the gauge G , this figure giving a measure of the gas space in tank A and being indirectly a measure of the volume of beer in the tank.

The volume of gas necessary to carbonate the beer to any degree of CO_2 content can then be calculated and in practice is introduced in the following manner. The tap Y is opened and gas drawn into the pump where the beer exists in a finely divided state with a large area *per* unit volume. Solution of the gas is therefore quite rapid. The gas pressure is allowed to fall to some convenient level (*e.g.*, 10 *lb. per sq. in.*), below which the rate of solution would become protracted. The taps are again closed, the tank M recharged to 30 *lb. per sq. in.* and the gas injected until the pressure is again lowered to 10 *lb. per sq. in.* This process is repeated until the requisite volume of gas has been dissolved. The initial pressure of the last charge of gas is so arranged that the final pressure is always 7 *lb. per sq. in.*, in order to avoid undue waste of gas. The rate of solution of carbon dioxide at the beer surface in the tank is so small that it has a negligible effect on the accuracy during the pressure measurements. The broad principles of the method are extremely simple, but before they can be applied on a practical basis, it will be obvious that a considerable amount of mensuration and arithmetic is necessary. Nevertheless, when this has been done, the figures can be collected into one simple table which can be used by an ordinary workman.

It will be appreciated that, in the above general description, the volumes of pipes a , b , c , d and e must be added to the volume of the tank M , and that the volumes of the pipes f , g and h outside the tank A together with the volumes of the pump and heat exchanger must be included in the volume of tank A .

With one exception, all the tanks of which

the volumes had to be measured were cylindrical in shape and were closed at either end by spherical caps. The volume was determined from the internal measurements of the axial length, the length of the straight cylindrical side and the mean diameter. The volumes of different tanks were checked against each other by filling one with water which was then forced by air pressure into another. The shortage or surplus was then measured.

According to Boyle's Law for a perfect gas, the product (total pressure $\times$ volume) remains constant under isothermal conditions. Amagat showed that this was not true for gaseous carbon dioxide, but that if the product be equal to 100 arbitrary units at 15°C. , the figure decreases by 0.0438 units with every *lb. per sq. in.* increase of pressure throughout ordinary brewery working conditions.

Let P *lbs. per sq. in.* = the gauge register of the gas tank.

p " " = the gauge register after connecting the tanks.

c cubic ft. = the volume of the gas tank.

v " " = the volume of the unknown gas space in the carbonating tank.

a *lbs. per sq. in.* = atmospheric pressure (an average figure is taken in practice).

k " " " = the constant after Amagat.

b " " " = $100 - ka$.

Then

$$\frac{c(P+a)}{100-k(P+a)} + \frac{va}{100-ka} = \frac{(c+v)(p+a)}{100-k(p+a)}$$

$$\text{i.e., } \frac{c(P+a)}{b-kP} + \frac{va}{b} = \frac{(c+v)(p+a)}{b-kp}$$

$$\text{i.e., } v = \frac{bc(P-p)}{p(b-kP)}$$

Let f *lbs. per sq. in.* be the initial pressure, and l *lb. per sq. in.* the final pressure in the carbonating tank. Then the volume (A) of any gas charge is given by:—

$$A = \frac{b}{a} \left[\frac{c(P+a)}{b-kP} + \frac{v(f+a)}{b-fk} - \frac{(c+v)(l+a)}{b-lk} \right]$$

Levelling charge.			2nd charge.		3rd charge.		4th charge.		5th charge.	
I.	L.	F.	I.	F.	I.	F.	I.	F.	I.	F.
30 → 19 → 10			→ 30 → 10		→ 30 → 10		→ 30 → 10		→ 14½ → 7	
30 → 18 → 10			→ 30 → 10		→ 30 → 10		→ 30 → 10		→ 13½ → 7	
30 → 17 → 10			→ 30 → 10		→ 30 → 10		→ 32½ → 7			
30 → 16 → 10			→ 30 → 10		→ 30 → 10		→ 32 → 7			
30 → 15½ → 10			→ 30 → 10		→ 30 → 10		→ 31½ → 7			

In the case of the first gas charge using the pressures we have suggested above, $P = 30$, $f = 0$, and $l = 10$. In the case of an intermediate charge, $P = 30$, $f = 10$, and $l = 10$. The volume of this charge will be found to be independent of the levelling pressure p and the volume v . In the case of the last charge, $f = 10$ and $l = 7$.

Above is an excerpt taken from one of the tables given to the foreman. The pressures are the readings of gauge G , indicating the pressure sequences in *lb. per sq. in.* taking place in tank M . Columns "I"

are the initial pressures and "F" the final pressures. In column "L" are given the levelling pressures.

The authors of this and the preceding paper would like to thank the Directors of Messrs. Joshua Tetley & Son, Ltd., for permission to publish these results.

The Brewery,
Leeds, 10.
December, 1948.

ABSTRACTS

I.—MATERIALS AND ANALYSIS

Take-All of Cereals in England and the Epidemic of 1948. W. C. MOORE (*Agriculture, London*, 1948, **55**, 383-385). Take-all was more widespread and destructive in England in 1948 than at any other time since it was first recognized (the distinct variety that occurs in Wales and the North-West was no more prevalent than usual). The disease is caused by a fungus which survives in the soil from one year to the next only in infected stubble residues and on the roots of certain weed grasses (see this *Journ.*, 1948, 270). Thus, provided these grasses are kept down, the fungus will disappear as the stubble residues rot, which takes about a year, so that a one-year break from wheat and barley is normally effective in checking take-all. Severe attacks are liable after a mild winter and a wet spring, and conversely little is seen of the disease after a cold winter and a relatively dry spring. The heavy rains of January, 1948, following the mild winter, therefore favoured the fungus, but the foundations of the epidemic were laid in the wet summer of 1946 (when take-all was widespread) and the severe winter that followed. Stubbles were not ploughed under until late in the spring of 1947 or, if they were, they remained frozen and unrotted in the ground. After the hot, dry summer which followed, the stubble was still unrotted in many districts when autumn sowings began, and this virtually nullified the effect of a one-year break from wheat and barley. The disease was checked by the dry weather later in the spring of 1948, but the wet summer which followed encouraged it again. The development of black mould on the bleached ears need not have caused the alarm it did, since it is a result and not a cause of disease. The experience of 1948 reveals the importance of giving a one-year break from wheat and barley at least every 2 or 3 years, and the uselessness of the break if susceptible weed grasses are allowed to grow. Early ploughing in of the stubble, a firm seedbed for the next cereal crop, and a generous top dressing with ammonium sulphate not later than March, are all measures which hamper development of the disease, and phosphate should be given when a second or third consecutive cereal crop is being grown. Oats are immune from the English take-all and can safely replace barley in the rotation, and seed grain harvested from an affected field can safely be used since take-all is not seed-borne (see also this *Journ.*, 1948, 158).

A. A. D. C.

Influence of Method of Harvesting on the Germinative Energy and Capacity of Barleys of the 1948 Crop. ANON. (*Brasserie*, Dec., 1948, 9). Of 62 barleys tested, 25 (representing 70 per cent. by weight of the total) had been harvested by combine and 37 by binder. The proportion of growers after 4 days' germination exceeded 90 per cent. in 20 of the combine harvested barleys and in 26 of the binder harvested barleys, but in 3 of the remaining 5 combined barleys 75 per cent. of the non-growers were dead, whereas in the remaining 11 binder harvested barleys only 29 per cent. of the non-growers were dead. The germinative energy was therefore greater in the combined barleys, but against this was the lower germinative capacity of 3 of the 25 lots. This fault is not inherent in the combine, however, but arises from improper storage of the grain, and the results so obtained on 1948 barleys show that combine harvesting can be carried out with advantage in a wet summer. Similar tests made in 1947 showed that the combine gives entirely satisfactory results under dry harvesting conditions.

A. A. D. C.

Use of Combine Harvesters in Belgium. ANON. (*Pet. J. Brass.*, 1948, **56**, 953-954). Some 30-70 per cent. of the losses of grain involved in the use of combine harvesters are due to bad regulation of the cutting blade. Correct setting must be determined by trials on one's own varieties. The machines in use in Belgium are designed for work in America and it is necessary to create a type adapted to the close, tall straws of European countries. At present, it is prudent to adopt a system of collective purchase and use, involving a group of farmers. Farmers using combine harvesters should sow cereal varieties resistant to lodging and varieties with high straw density should be replaced by those listed as "average." Choice of each cereal sown should be made with a view to spreading the harvest. Other important associated problems consist in (1) collection of threshed straw; (2) drying the grain; (3) storage of the dried grain. It is essential that all grain driers should be equipped with a moisture-determining apparatus. The author believes that the number of privately owned combine harvesters in Belgium will not increase much because of the uncertain climate.

C. R.

Storage Drying of Grain. R. A. VINTNER (*Agriculture, London*, 1949, **55**, 488-489). Experiments have been carried out over a period of three years with a grain drier using a

forced current of air, and successful results have been obtained with combine-harvested grain, including wheat, barley and oats with an initial moisture content of upwards of 25 per cent. Drying is carried out in vertical towers built in sections, the bottom section of expanded metal covered with asbestos being provided with twin hoppers of perforated sheet steel, the perforations allowing entry of air but being too small to allow grain to fall through; this bottom section is surmounted by three tiers which might be constructed, *e.g.*, of aluminium sheet. A tower so built holds 100 quarters, and can be dismantled and re-erected at will, a suitably overhanging lean-to roof providing sufficient cover. The tower is filled by means of a pneumatic portable grain elevator and the blower fan is put on when the hopped section is full, the air being electrically heated to 12–15° F. above the temperature of the outside air. Operational supervision is reduced to a minimum and, if turning is required, this is done by circulating with the elevator. A check on the moisture content of the grain is required after about 4 days. For emptying, the ventilating fan is used for transferring the grain to a sacking-off hopper. The average cost of drying (including filling) is 18s. to 20s. per ton of grain dried. A sample of S147 oats dried for 5 days in the way described contained 14 per cent. of moisture and showed 98 per cent. germination

I. A. P.

Oxidizing Enzymes in Brewing Materials. V. Peroxidase in Malt Adjuncts. J. S. WALLERSTEIN, M. G. HALE and R. T. ALBA (*Wallerstein Lab. Commun.*, 1948, 11, 319–322). Peroxidase activity, previously studied in malt and barley using *o*-phenylenediamine as substrate (this *Journ.*, 1948, 220), has now been studied in wheat, rice, and white and yellow maize. In raw grain, the activities increase in the order rice, maize, barley, wheat; during malting, considerable increase in activity is developed, the final activities being maintained in the same order of increase. Particularly high concentration of the enzyme occurs in rootlets and acrospire in all cases. 68–76 per cent. of the activities of malted grains persists after kilning, whilst 35–52 per cent. survives experimental mashing. A p_H value of 4.6–5.0 is optimal for activity from each source.

I. A. P.

Specific Gravity Gradient Tube for Brewery Control. L. ATKIN, I. STONE and P. P. GRAY (*Wallerstein Lab. Commun.*, 1948, 11, 281–288). The gradient tube originally described by Linderstrøm-Lang and Lanz has been tested for its utility in brewery process control. A tall graduated jar (*e.g.*, a 500 ml.

cylinder) is required, and 275 ml. of a solution of bromobenzene in kerosene is poured in, this solution being of such concentration that its sp.gr. is greater than the maximum required. A second solution of bromobenzene in kerosene with a sp.gr. less than the minimum required is poured carefully on top to form a layer, and graded mixing is brought about by careful manipulation of a wire spiral on a long wire handle, the movement of the spiral at first being through a limited range around the interface, but through a progressively increased range thereafter. Thus, a gradient of sp.gr. is produced down the cylinder. For brewery work, three gradient tubes are recommended, having sp.gr. ranges respectively of 1.004–1.028, 1.020–1.040, and 1.032–1.064. The sp.gr. of an unknown solution is estimated by comparing the position a sample drop takes up in the appropriate tube with the positions attained by two drops of standard solutions of known sp.gr., one standard being lighter and the other heavier than the unknown. Standard solutions are conveniently prepared from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and fresh standards should be prepared monthly. When an observation is complete, the tube is cleared by shaking on from a sprinkler a little clean sand or, better, sodium chloride, the sprinkled material falling through the tube and carrying down the drops with it to the bottom. Carrying out 15–20 observations daily, the gradient tubes appear to have a useful life of about 3 months.

Comparison of results for beers, worts and syrup solutions by this method with those obtained by the pycnometer shows very small differences, which though apparently significant are unimportant in practice; the differences may be due to surface tension effects producing, from a standard dropper, drops of slightly different size with different solutions. Precision is probably greater than when a saccharometer or hydrometer is used. An interesting application of the method is its use in following the rate of attenuation in fermenting laboratory worts.

I. A. P.

Distribution of Thiamine and Riboflavin in Wheat, Oats and Barley at Successive Stages of Plant Growth. A. D. ROBINSON, L. E. LYND and B. J. MILES (*Canad. J. Res.*, 1948, 26B, 711–717). To test the effect of time of harvesting on the thiamine and riboflavin content of certain cereals, Renown wheat, OAC21 barley and Vanguard oats were grown on experimental plots. Samples for analysis were taken roughly every week and were prepared from 150 plants taken at random, which were separated from the roots at ground level, cut up, mixed, and duplicate samples for each analysis taken from the mixture. Thiamine was determined by Hennessy's

method and riboflavin by Andrew's fluorimetric procedure (this *Journ.*, 1944, 51).

In wheat, barley and oats the thiamine content of the plant rises until heading takes place, after which the vitamin is translocated into the kernels, which contain 80-90 *per cent.* of the total at maturity, the remainder being located in the stem and, in barley, in the glumes and rachis, negligible amounts being found in the leaves.

Riboflavin is produced early in the life in all the grains. Some time after emergence the vitamin content reaches a peak, thereafter falling as the plant matures. In wheat, the content of whole plant and of kernel is constant from maturity onwards, the kernel contributing 28 *per cent.* of the total and the stems 55 *per cent.* The total riboflavin of barley continues to fall even after maturity, but the content of the kernels is constant from maturity, being about 40 *per cent.* of the total, with a roughly equal amount in the stem. Oats resemble barley, except that the content of the kernels rises onwards from maturity. In all three cereals, appreciable riboflavin is found in the leaves.

C. R.

Determination of Dextrins in Worts and Beers. J. DE CLERCK and G. VAN ROEY (*Bull. Assoc. anc. Etud., Louvain*, 1948, 44, 105-123). After discussing the constituents of wort and beer which are liable to interfere with the determination of the dextrins, the authors review and criticize the methods which have been put forward by others, pointing out that the two main obstacles to accuracy are the lack of homogeneity of the dextrins and their persistent contamination with small amounts of other substances. The method they propose is an enzymic one. To 25 c.c. of beer or 20 c.c. of wort are added 5 c.c. of a 2 *per cent.* amylase solution and 2 c.c. of sucrase (invertase) solution. After adding a few drops of toluene the flask is closed and kept at 55° C. (131° F.) for at least 48 hours with occasional shaking. The liquid is then cooled, made up to 200 c.c. volume, and the copper reducing power determined on an aliquot. A duplicate test is carried out omitting the amylase. The difference in copper reducing power between the two is calculated as maltose, and this figure multiplied by 0.95 gives the dextrin content of the sample. The only error involved is due to the reducing power of the dextrins. This is small, and a correction can be derived if needed. Care must be taken that the sucrase solution contains no reducing sugars; bakers' yeast as a source is satisfactory, but brewers' yeast must be exhaustively washed with water and borax before its treatment with ether-alcohol. Results given by this method are much lower than those

given by acid hydrolysis, and the authors substantiate their own in two ways. Dextrins precipitated from the fully attenuated sample, redissolved, and hydrolyzed with acid gave approximately the same results, as did computation of the dextrins by difference. A detailed account is given of attempts that were made to isolate the dextrins from beer and wort quantitatively and in a pure condition, and from successive alcohol fractionations and fermentations it is concluded that the dextrins possess a small reducing power of their own. Precipitation of the dextrins appeared to be complete when alcohol was added to a concentration of 90 *per cent.*, but the dextrin was found to be slightly soluble in alcohol. In addition, the precipitated dextrins always entrained a small amount of reducing sugars from the wort or beer, even if nitrogenous and colouring compounds were removed by a preliminary treatment of the sample with adsorbent carbon.

A. A. D. C.

Electrical Determination of Moisture in Grain. TRIOEN (*Pet. J. Brass.*, 1948, 56, 980-981). The theoretical bases of four electrical methods, directly applicable to the determination of moisture in cereal grains, are described. (1) The grains are placed in an insulated cylinder between two metal plates. The system so formed is a condenser whose rate of discharge or recharge can be measured, the rate increasing with the moisture content of the grains. (2) Using the same cylinder system as in (1), if a high frequency alternating current is applied to the plates, a temperature increase is observed which is proportional to the dielectric loss, the latter being directly proportional to the moisture content of the grains between the plates. (3) Measurement of the capacity of a condenser, the dielectric of which is formed by the grain under test, may be made by a Wheatstone's bridge circuit. The capacity varies with the moisture content of the grains and for each type of cereal the apparatus must be standardized. A valve voltmeter or "magic eye" indicator may be used for determining the point of balance of the bridge. (4) A non-aqueous hydrophile electrolyte may be used to extract water from grain, the process resulting in a sharp increase in conductivity. The ground grain is extracted, usually with acetone containing dissolved oxalic acid, and the increased conductivity of the extract observed. All operations must be carried out under conditions which prevent evaporation of acetone.

C. R.

Determination of Inorganic and Organic Phosphate in Plant Extracts. E. R. WAY-GOOD (*Canad. J. Res.*, 1948, 26C, 461-478). The author has applied the method of Lowry

and Lopez for determining the phosphate radical to ascertaining the amount of inorganic and organic phosphorus in plant extracts. In the original method, ascorbic acid is used as the reducing agent for the production of a blue colour by its action upon the phosphomolybdate formed. The method has been re-investigated and the effect of the various factors determined, the results in general confirming those of Lowry and Lopez. The use of ascorbic acid is preferable to the reducing agents formerly used, since the full development of colour is complete in about 5 minutes and then remains stable for a considerable time. Details are given of the conditions yielding the most satisfactory results, the final colour intensity being determined at a wave-length of 650 m μ . In general, the material was finely ground and the juice separated, filtered, deproteinized with trichloroacetic acid and centrifuged, after which the inorganic phosphorus was determined directly. A wet combustion of the juice with perchloric acid was used for measuring the total phosphorus, whilst hydrolysis with N hydrochloric acid at 100° C. of different portions for 7, 60 and 180 minutes was used for ascertaining the various types of organic phosphorus present.

T. J. W.

Silica and Tannin in Worts and Beers.

I. STONE and P. P. GRAY (*Wallerstein Lab. Commun.*, 1948, 11, 301-318). Silica has long been known to be present in brewery raw materials, and although only a small proportion of that present in malt passes into the wort on mashing, silica is present in many beer hazes. The classical gravimetric methods for determining silica are unsatisfactory with beer and wort, and a colorimetric technique has been developed for use both with degassed beer and with wort pretreated with tannic acid to remove protein. The liquid in either case is then treated with barium chloride in presence of potassium hydroxide to precipitate phosphate, after which ammonium molybdate solution is added and a freshly prepared solution containing 1-amino-2-naphthol-4-sulphonic acid with sodium bisulphite and sulphite. The colour intensity, measuring the silica present, is read photoelectrically, a reagent blank having been similarly conducted on silica-free water; different calibration factors are required for beer and wort. Samples of beer and wort examined contained 50-90 p.p.m. of silica and recovery of added silica was 97-105 per cent.

The importance of tannin in brewing has long been a matter of discussion, but determination is complicated by the variable compositions of tannins from different sources and, as in the present case, tannic acid is usually taken as a standard of reference. In the photometric method now adopted, the solution under test

is treated with ferric ammonium citrate in presence of gum arabic; dilute ammonium hydroxide is added and the colour reading is determined. Controls are necessary: (i) as above, but without the iron reagent; (ii) using distilled water instead of the sample and both with and without added iron. From the data obtained and using a calibration factor established with tannic acid, tannin can be calculated. If the sample contains initially more than 0.5 p.p.m. of iron, results may need correction. Three samples of beer examined contained 31-43 p.p.m. of tannin; recovery of added tannin was 93-113 per cent.

Using these methods, it was shown that, derived from husk and being increasingly extracted during sparging, silica shows little change in amount during the brewing process; tannin is derived both from malt (without enhanced extraction during sparging) and from hops. Dissolution of silica from bottles does not appear to occur, and addition of silica up to 2-3 times its normal concentration to beer does not accelerate haze development. Silica determination in beer indicates the extent to which associated husk constituents may have been extracted, a matter of importance in view of the influence these may have on flavour and colour.

I. A. P.

Colorimetric Determination of Iron with Isonitrosodimedon. S. C. SHOME (*Analyt. Chem.*, 1948, 20, 1205-1208). Spectrophotometric studies of the reaction between iron and isonitroso - dimethyldihydroresorcinol (isonitrosodimedon) indicate that the reagent is useful for the colorimetric determination of iron. The reagent is readily prepared from dimedon by treatment with nitrous acid. Estimations were carried out with freshly prepared 0.1 per cent. aqueous solutions of the reagent reacting at p_H 4-5 in presence of ammonium acetate. Comparisons by means of a Dubosq colorimeter were made between standard ferrous and ferric iron solutions containing respectively 0.02 and 0.025 mgrm. of the ions per ml. and samples not differing by more than 25 per cent. in iron content from the standard. The method will detect 1 part of iron per 50,000,000 parts of solution. The reagent reacts with both ferrous and ferric ions giving a stable, blue complex containing 3 molecules of isonitrosodimedon to each atom of iron, so that the colour produced is a function of the total iron present. The method is unsuitable for the determination of ferric iron in presence of such anions as phosphate, arsenate, fluoride, cyanide and oxalate which form stable complexes with ferric iron. Copper cobalt and nickel give coloured systems with the reagent, the maximum permissible limits

of these ions in a determination being, respectively, 2,150 and 180 *p.p.m.* Cr^{+++} in excess of 20 *p.p.m.* also interferes by reason of its own colour. The sensitivity of the method is as high as that of α , α' -dipyridine or *o*-phenanthroline.

C. R.

II.—FERMENTATION

Sporulation in Yeasts. Part I. H. J. PHAFF and E. M. MRAK (*Wallerstein Lab. Commun.*, 1948, 11, 261–279). Study of the formation and characters of ascospores is fundamental in yeast classification, the spore-forming or “perfect” yeasts being grouped on the Stelling–Dekker system in the family *Endomycetaceae*, whilst non-sporulating forms are ascribed to *Torulopsidaceae*, *Rhodotorulaceae*, or *Sporobolomycetaceae*. Considerable advances have been made recently in studying the cell fusions which occur before or after spore formation, and a number of different types of behaviour can be recognized. In *Type 1*, the vegetative cells are characteristically haploid (*i.e.*, in the n chromosome condition), and the short-lived diploid phase (the $2n$ condition) results from the fusion of two haploid cells immediately before sporulation. This is found, *e.g.*, in *Endomyces* and in some species of *Schizosaccharomyces*. Vegetative cells in *Type 2* are diploid; the haploid condition is confined to the spores themselves and possibly a limited number of budded generations thereafter, fusion soon leading to a return of the diploid state. Such behaviour is characteristic of *Saccharomyces*. *Type 3*, perhaps of limited importance, shows the mother cell conjugating with an attached daughter cell, one or two spores forming in the zygote. *Type 4* somewhat resembles *type 2*, but spores conjugate directly in the ascus, as occurs in *Saccharomyces cerevisiae*. *Type 5*, found in *Nadsonia*, shows fusion of mother and daughter cells, the contents of the zygote passing into a second bud which acts as ascus. The diploid vegetative cells of *Type 6* give rise to buds in which ascospores are produced, these budding to form binucleate vegetative cells. The type of behaviour is not fixed for a particular genus, and even within a species variations may occur.

The shapes of the asci vary considerably from one genus to another, from the ellipsoidal or globular asci of most *Saccharomyces* to the ellipsoidal or elongated form often found in chains in *Pichia*. Shape, number, and surface characters of spores are of considerable importance in differentiating genera and species. The number of spores in true yeasts does not usually exceed eight, but in a few cases as many as 32 are found; in most yeasts the number is 1–4, the latter being the most usual. A variety of spore shapes is encountered: spherical

to globose (most *Saccharomyces*), kidney-shaped (*S. fragilis*), sickle-shaped (*Endomycopsis selenospora*), hat-shaped (*Hansenula*), spindle-shaped (*Nematophora*). Smoothness, the presence of a rim, or of warts, are other diagnostic features, whilst it is also of interest to observe whether spore germination takes place in the ascus (so rupturing it) or whether spores are liberated immediately after formation, though such behaviour is not constant. In studying germination, confusion with the behaviour of vegetative cells must be avoided and the relative resistance of spores to 50 *per cent.* alcohol may be made use of, vegetative cells being rapidly killed by this reagent. Although it is generally held that spores are relatively resistant to adverse conditions, the comparative behaviour of vegetative cells and of spores towards temperature is not always so straightforward as might be expected. For the differential staining of spores, the method given by Shimwell (this *Journ.*, 1938, 474) is simple and satisfactory.

I. A. P.

Estimation of Yeast Concentration by Means of the Specific Gravity Gradient Tube. L. ATKIN, M. FEINSTEIN and P. P. GRAY (*Wallerstein Lab. Commun.*, 1948, 11, 289–300). In the case of aqueous suspensions of washed brewery yeast, the sp.gr. determined by the gradient tube (see page 117, this issue) is linearly related to the concentration of yeast solids (Y); a “yeast modulus” (M) is defined as the increment of gravity [1000 (sp.gr. – 1)] produced by 1 gm. of yeast solids *per* 100 ml. of suspension. The average value of M is 3.21 for washed yeast and 3.44 when the yeast is suspended in its own beer. For yeast suspensions in beer, as with liquid brewery yeast, the gravity of the beer (b) and a “cell constant” (K) also influence the linear relationship between the gravity of the suspension (G) and Y , the relationship being given by: $G = Y(M - b/K) + b$. The cell constant is defined as the grms. of yeast *per* 100 ml. of yeast cells, and an average value for it is 40.87. Using this relationship, the concentration of solids *per* 100 ml. can be calculated to within 2 *per cent.* of its value; *e.g.*, with a suspension containing 15 *per cent.* of yeast solids to within 0.3 *per cent.* Further, it can be shown that the yeast cell itself contains 36.13 *per cent.* of solids, agreeing closely with the value of 36.4 *per cent.* established by Montgomery and White (this *Journ.*, 1945, 279). However, it is realized that the 10 yeasts studied in the present work may not be truly representative of all yeasts, and for accurate work it might be necessary to establish individual values of M for different yeasts, if the gravity method is to be used for determining yeast concentration.

I. A. P.

Some Factors Affecting Growth and Nitrogen Structure of a Pure Culture *Saccharomyces cerevisiae*. W. M. CLARK (*Amer. Brewer.*, Dec. 1948, part I, 25-29, 62-63). Over a period of 30 months the total nitrogen of the unchanged pure yeast used in a brewery was found to vary by as much as 10 *per cent.* In general, with increase in total nitrogen the amide, humin and mono-amino nitrogen also increased whilst the non-amino and dibasic nitrogen decreased. There were concomitant variations in fermentation and yeast sedimentation, although the brewing process was unchanged throughout. In an attempt to learn the cause of the variations in yeast composition, the author has developed a culture from a single cell of the yeast (a Froberg type, bottom fermentation) and grown it in a basal Laurent's medium to which had been added different sources of carbon and nitrogen to simulate wort from (1) an undermodified, high nitrogen malt, (2) an undermodified, medium nitrogen malt, (3) an overmodified, medium nitrogen malt, and (4) a malt undermodified in regard to carbohydrate but overmodified in regard to nitrogen. The effects of a number of inorganic salts were also tested. Of the 25 media so prepared, 8 contained one amino acid each, 1 contained urea, 8 had one inorganic salt each, 5 were made with varying concentrations of carbohydrates and 3 contained peptone. Starting with these cultures, daily transplants were made into their respective media for 120 days, at which time they were all inoculated into wort and once again transplanted daily for 240 days. On the 10th and 120th days, inoculations of the wort cultures were made into 1 litre amounts of their respective media, and on the 240th day into 1 litre amounts of wort. Growth in the litre cultures was continued until the sugar had been used up, and the yeasts were collected and analyzed for nitrogen distribution according to Hausmann's classification (amide, humin, dibasic, mono-amino and non-amino nitrogen). The fermented liquors were also analyzed. From the mass of data which the author presents it is only possible to quote the broad conclusions, as follows. The nitrogen content of the yeast (dry weight) is increased by increasing amounts of carbohydrates and by increase in the amount of assimilable amino nitrogen. Certain inorganic salts cause a permanent decrease in the yeast nitrogen, others cause at least a temporary increase. Alcohol production increases as total yeast nitrogen decreases. This decrease takes place at the expense of the non-amino, humin and amide nitrogen; dibasic nitrogen is practically unchanged, whilst the mono-amino nitrogen actually increases.

A. A. D. C.

Influence of Proteolytic Enzymes and Yeast Nutrients upon Requirement for Malt in Grain Alcohol Fermentations. J. M. VAN LANEN, E. H. LEMENSE, A. ANELLIS and J. CORMAN (*Cereal Chem.*, 1948, 25, 326-336). This work was undertaken to find whether the amount of barley malt used in an alcohol distillery—normally 8 to 10 *per cent.* of the total grain—could be reduced without sacrificing yield of alcohol or unduly prolonging fermentation, and the use of proteolytic enzymes, protein hydrolyzates, vitamins and other yeast growth factors to replace part of the malt has been investigated. The procedure followed was to pre-malt slurries of maize or wheat at 70° C. (158° F.) for 30 mins. with malt equivalent to 1 *per cent.* of the grain bill, then to cook the mash at 25 lb. pressure for 30 mins. The cooked mash was cooled and varying qualities of conversion malt and malt adjuncts (protein hydrolyzates, *etc.*) were added. After holding at 58-60° C. (136-140° F.) for 30 mins., the temperature was lowered to 30° C. (86° F.) and 2 *per cent.* of distillers' yeast was added. The effect of the adjuncts upon both rate of fermentation and final yield of alcohol was followed. Papain, ficin, culture filtrates from *B. subtilis*, and protein hydrolyzates were found capable of replacing a portion of the conversion malt. When these were supplied at their optimum concentration, equivalent yields of alcohol and rapid fermentations were obtained with 60 *per cent.* of the normal amount of malt. There is evidence that different protein hydrolyzates vary in their effectiveness according to the nature of their constituent amino acids; also, that these constituents may be absorbed in substantial amounts by seed yeast, since a beneficial effect was produced by adding the adjuncts to yeast seed cultures. It was shown that the effect of proteolytic enzymes is associated with nutrition of the yeast rather than with the release or activation of malt amylases, and it is suggested that a similar result might be achieved by the use of malts of greater proteolytic activity, or by adding assimilable nitrogen to seed yeast media.

A. A. D. C.

Production of 2, 3-Butanediol from Barley. R. V. TOMKINS, D. S. SCOTT and F. J. SIMPSON (*Canad. J. Res.*, 1948, 26F, 497-502). Barley mash containing 12.5 *per cent.* or more of grain were inoculated with cultures of *Aerobacillus polymyxa* after the addition of sterile calcium carbonate, and fermentation was allowed to proceed at 32° C. for 96 hours in a pilot plant. From the fermented mass the ethyl alcohol was removed by distillation and the remainder was filtered,

concentrated by evaporation, precipitated by the addition of alcohol and after removal of the solids the alcohol and butanediol were separated by steam stripping. Data are given of the fermentation efficiency, the cost of plant, energy used, raw materials and the yield and value of the products. Owing to the coarse hulls of the barley blocking the mash pipeline trouble occurred; this was overcome by streamlining the pipe. Although barley contains less starch than wheat the cost of production of the butanediol was lower when the former was used as source. T. J. W.

III.—BEER

The Concept and Nature of Redox Potential. K. RIPPEL (*Brauwissenschaft*, 1948, No. 3; through *Brewers Digest*, 1948, No. 12, 43-45, 49). The redox potential or r_H value of a liquid indicates the relationship between the oxidising and reducing substances present and ranges from 0 to 42, the former being equivalent to a hydrogen pressure of one atmosphere and the latter to the complete absence of hydrogen. The actual figure for r_H is the negative logarithm of the hydrogen pressure in solution. Oxidation or an increase in r_H involves either a gain in oxygen, a loss of hydrogen or an increase in the positive charge on a substance, whilst reduction is a reversal of these actions. Since each r_H value corresponds to a definite electrical potential, a measurement of the latter may be used to determine the required value. In addition, determinations may be made by means of indicators (usually dyes) which change colour at definite r_H values. Most biological liquids contain numerous substances which have reducing or oxidizing properties together with others which act as catalysts and thus accelerate the actions of the preceding. Numerous examples of such substances both inorganic and organic are given in the paper. Since r_H is affected by the presence of oxygen and also by the p_H value of the solution, oxygen must be eliminated and the p_H value considered when a determination is being made. Bacteria require for their development a definite short range of r_H value and for anaerobes this is close to 0, whilst aerobes favour higher values of 25 or more. Yeasts are adaptable to both low and higher values but the former induce fermentation and the latter favour respiration and reproduction. Wort has an r_H value of about 25 but during fermentation this is reduced, and beer shows a value of 11 or 12. The stability of beer both in freedom from haze and quality of flavour is enhanced by a low value and for this reason the finished beer should be kept out of contact with air. T. J. W.

IV.—PLANT, MACHINERY AND BY-PRODUCTS

Improving Bottling Plant Operation. N. G. RHODES (*Amer. Brewer*, Dec. 1948, part 1, 46, 48, 58). There is more room for development and improvement in bottling than in any other brewing process, but in order to take advantage of the possibilities it is essential to know the efficiency of an existing plant, and how it compares in efficiency with other types of plant. This knowledge can be acquired only through a system of control of every stage of the process and by keeping full operational records. Even then comparison of two types of plant is difficult, for there is no generally accepted method of keeping bottling records. The need for such a method is obvious, and it must be so planned as to require no significant changes over a period of years or it will not be possible to compare present results with those of earlier years. The author gives a sample record sheet as a suggestion of what is needed. The reluctance that may be felt by some bottlers to disclose material facts to outsiders may be overcome by using percentages instead of the actual figures in their records for the purposes of comparison. A. A. D. C.

Detergent Action. O. C. BACON and J. E. SMITH (*Industr. Engng. Chem.*, 1948, 40, 2361-2370). The work described was carried out in an attempt to measure directly, by a washing procedure, the amount of work done by a detergent, and so to compare detergents on a direct and practical basis. The variables studied were the detergent, the mechanical force, and time; temperature and ease of soil removal were kept constant and soil redeposition was avoided by the experimental conditions. A combination of lamp black, a mineral oil, a hydrogenated vegetable oil and starch comprised the standard soil, and the amount of work was measured by using a standard washing machine. The *per cent.* of soil removal was calculated from reflectivity measurements on the test fabric before and after detergent treatment, by reference to a series of curves relating soil removal to reflection. The curves were constructed by measuring reflection photometrically against a standard magnesium oxide surface, and determining *per cent.* soil removal from a knowledge of the reflectivities of the unsoiled fabric, the soiled fabric, and the cleaned fabric. Over the range of detergent concentration and mechanical work studied, and where—as in this work—increased detergent is accompanied by increased soil removal, it has been shown that (1) the concentration of detergent required is inversely proportional to the mechanical force used when degree of soil removal, time and temperature

are kept constant; (2) the time is inversely proportional to the detergent concentration when degree of soil removal, force and temperature are kept constant; and (3) the time is inversely proportional to the force when degree of soil removal, concentration and temperature are kept constant. An equation relating detergent action with concentration, force and time has been derived for a given temperature under conditions where increased detergent concentration is accompanied by increased soil removal.

A. A. D. C.

Past, Present and Future of Diatomaceous Silica. F. L. HORINE (*Brewers Digest*, 1948, No. 11, 43-44, 46). The demand for diatomaceous silica (kieselguhr or diatomite) for filtration purposes in the American brewing industry exceeds 1000 tons a month, this being used principally for beer and a small proportion only for wort. Kieselguhr consists of the fossilized shell-like remains of microscopic plants and at death the shells accumulate on the ocean floor forming layers sometimes 1000 ft. thick which are later raised above sea level by natural agencies. These shells consist entirely of silica and occur in a variety of shapes and sizes, the average length being about 1/2000 in.; a volume of 1 cu. in. will contain about 35 million. About 1870, filter trials with kieselguhr were carried out in Germany but for various reasons unsatisfactory results were obtained. Little progress was made until about 1920, when two American brewers investigated the subject, determined the most suitable varieties, and improved the quality by physical and chemical treatments. Kieselguhr is now obtainable in several particle size grades suitable for either coarse or fine filtration, the latter removing substances in a colloidal condition from beer although the rate of flow is less than when coarser grades are used. When employed as a filter-aid, kieselguhr is mixed with the beer before filtration and causes the deposit of yeast, *etc.* on the pulp or fabric to be porous, thus facilitating filtration and ensuring a longer run before breaking down. Different brewers use filtration at different stages of the brewing process; a few filter the wort before it enters the fermenting vessel in order to keep the yeast clean, give a shorter fermentation time, and a cleaner aroma and flavour to the finished beer; others use the filter immediately after fermentation and before storage and thus improve the quality and stability of their product. When kieselguhr was first used on a large scale in the brewing industry, little was known about the material or the most suitable filter either by the brewer or the filter maker, and some of these earlier types are still in use with more or less indifferent results. It is essential for real success

that different parts of the filter plant should all be of proper size and in a definite ratio to each other, and an investigation of clarification equipment might prove of value. T. J. W.

Heating and Cooling Economics of the Beer Pasteurizer. W. NEKOLA (*Amer. Brewer*, Nov., 1948, 24). Tests were made on a spray-type pasteurizer, in which heating was effected by the injection of live steam into the water. The values of the specific heat and weight of bottles, beer, *etc.*, were used to determine specific heat weight constants, and during the tests readings were made of all data possible, such as steam used, temperatures, number of bottles pasteurized, *etc.* The results obtained indicated that 73 *per cent.* of the total heat was used for heating the beer, 7 *per cent.* was lost in condensate overflow, and 20 *per cent.* disappeared by radiation and other miscellaneous losses. With different sizes of pasteurizer approximately one boiler horse power was required to pasteurize 1 American barrel (26 Imperial gallons) of beer. Cessation of work during lunch time when the pasteurizer is emptied and refilled later may require about 11 *per cent.* more steam than continuous operation. At the present day a standard pasteurizer using water at 65° F. and discharging the beer at 85° F. may require only $\frac{1}{3}$ to $\frac{1}{2}$ the usual quantity of water if an additional cooling section is provided. The actual cost of steam, water and power for operating a standard machine working efficiently is about 1½d. per barrel of beer pasteurized. The spray pasteurizer is more economical of labour than any other type and necessitates much less expenditure on repairs. Owing to recent improvements in machines and bottles the loss due to breakage is on an average 0.02 *per cent.* when 12 oz. bottles are used. Several varieties of heating are available utilizing high, medium or exhaust low pressure steam, and either may be best suited to the conditions of any particular bottling.

T. J. W.

Lowering Production Costs by Use of Heat Insulation. U. W. SMITH (*Amer. Brewer*, Oct. 1948, 21-24). The author describes the adverse effects of inefficient heat insulation of brewery plant not only in actual loss of heat but also in the formation of an excessive amount of wet steam, which causes rust deterioration, scoring of propeller blades, and power losses. He also exposes the fallacious beliefs that outer surface temperature of the insulation is an indication of its efficiency and that an air space between a hot surface and its insulation prevents loss of heat. The ideal insulating medium would be a vacuum and the next in efficiency is "dead" or non-circulating

air contained in the small closed spaces of a porous material. For temperatures up to 600° F. magnesia reinforced with asbestos has many advantages, but for higher temperatures the same material mixed with kieselguhr is used. Details are given of the thickness of insulating material required for different parts of brewery plant from the points of view of efficiency and economy. The best method of applying and protecting insulation is described and also the necessity for insulating flanges, steam cocks, and other fittings. To ensure successful results, regular inspection of all insulation should be carried out in order to detect and remedy any damage, loosening or breakdown. A detailed table is provided showing the monetary saving resulting from the use of magnesia insulation on pipes of different diameters conveying steam at various pressures. T. J. W.

V.—BIOCHEMISTRY

End-Group Determination of Amylose and Amylopectin by Periodate Oxidation. A. L. POTTER and W. Z. HASSID (*J. Amer. chem. Soc.*, 1948, **70**, 3488–3490). By treatment with periodate, the ring of methyl hexopyranosides is broken and the third carbon atom eliminated as formic acid. Each non-reducing polysaccharide end-group yields one mol and each reducing end-group two mols of formic acid. Hirst *et al.* (this *Journ.*, 1948, 282) have utilized the periodate oxidation method to determine the number of glucose residues per non-aldehydic end-group of starch. The present work was designed to find conditions of overcoming the difficulty of over-oxidation by periodate. Those fixed upon consisted in treating a known amount of polysaccharide in 3 per cent. sodium chloride solution with sodium metaperiodate at 2° C. for 25 hours, after which excess reagent was decomposed by standing with ethylene glycol in the dark for 1 hour at room temperature. Formic acid was determined by titration with 0.01 N baryta using methyl red as indicator. From this the chain length was determined by calculating the number of grms. of the polysaccharide corresponding to 3 mols of formic acid and dividing by the molecular weight of anhydroglucose (162). Amyloses from starches of six different plants gave chain lengths of 420 to 980 glucose residues and the corresponding amylopectins 22 to 27.

The periodate oxidation method is less time-consuming than the standard methylation practice for end-group determination and eliminates the possibility of degradation of the starch molecule which may occur during methylation. C. R.

Molecular Weights of Amyloses and Amylopectins from Starches of Various Plant Origins. A. L. POTTER and W. Z. HASSID (*J. Amer. chem. Soc.*, 1948, **70**, 3774–3777). The investigation was carried out to determine the molecular weights of the amylose and amylopectin components of starch under conditions of minimal degradation. Owing to the retrogradation of amylose, and the highly colloidal nature of amylopectin in aqueous solution, acetylated derivatives dissolved in an organic solvent were selected for molecular weight determination. Acetylation was carried out by treatment of a formamide dispersion of the starch fraction with acetic anhydride and pyridine at room temperature, the treatment being repeated a second time to complete the acetylation. Molecular weights were determined by osmotic pressure measurements of the acetylated products in chloroform solution. Since most polymers consist of an assemblage of molecules of different molecular weights, "number-average" values were obtained. Those obtained for six amyloses from different plant sources varied between 100,000 and 210,000, and for amylopectins between 1 and 6 millions. Comparison of these figures with chain-length values obtained by periodate end-group determinations (see preceding Abstract) show that the amylose molecules of potato and Easter lily starches consist of single chains, whilst, in other cases, two or three chains may be linked in one amylose molecule, indicating the possibility of a slight degree of branching. C. R.

Effect of Acid Hydrolysis on the Retrogradation of Amylose. R. L. WHISTLER and C. JOHNSON (*Cereal Chem.*, 1948, **25**, 418–424). The information given is of use to workers requiring to keep starch solutions with minimal retrogradation. The amyloses used were prepared by butanol fractionation of maize, wheat and potato starches. Solutions were prepared by adding the amylose-butanol paste to boiling water, stirring at 95–98° C. to vaporize the butanol and adjusting to 1 per cent. concentration. Hydrolyses of these solutions were carried out for definite periods at 96° C. in presence of 0.005 N sulphuric acid. The amount of retrogradation of a sample was determined as the difference between the initial amylose concentration and that determined on the supernatant liquid obtained after standing 24 hours at 25° C. and centrifuging off the retrograded amylose.

The rate of retrogradation of potato amylose from 0.85 per cent. aqueous solution is markedly affected by acid hydrolysis. Maximum rates are observed after 30 to 60 minutes hydrolysis. At the same time, molecular weight, as evinced by viscosity measurements, decreases rapidly.

Maize amylose shows a similar maximum. The unhydrolysed amyloses of maize and wheat retrograde more rapidly (75.4 and 86.7 *per cent.*, respectively) than potato amylose (16 *per cent.*), but not so rapidly as the latter after hydrolysis for 30 to 60 minutes (94 *per cent.*). The results indicate that there is a critical molecular size at which amylose retrogradation is maximal: larger molecules retrograde more slowly because of steric effects, and smaller ones because of the increased solubility inherent in lower molecular weight.

C. R.

X-Ray Molecular Weight of β -Lactoglobulin. F. R. SENTI and R. C. WARNER (*J. Amer. chem. Soc.*, 1948, **70**, 3318-3320). Physical methods of molecular weight determination of proteins in solution are subject to uncertainty due to aggregation or degradation. This uncertainty is minimized by use of the X-ray diffraction method applied to proteins in the native crystalline state, since any denatured protein does not contribute to the sharp interferences given by the native protein. X-ray data give a direct estimate of the volume of an integral number of molecules of native protein. The value of this number can be decided from a knowledge of the space group of the crystal (also given by X-ray data) and of the approximate molecular weight derived from osmotic pressure or ultracentrifuge methods, so that the volume of the protein molecule can be measured. From this volume, and the values of the density and water content of the protein crystal, the anhydrous molecular weight can be calculated.

Crystalline β -lactoglobulin was prepared by fractionating whey with ammonium sulphate at p_H 5.8-6.0. The fraction soluble in 2.2 M, but insoluble in 3.3 M $(NH_4)_2SO_4$ was dialysed at p_H 5.2 and the protein so obtained recrystallized four times by redissolving in dilute NaCl and dialysing. The hydration of the crystals was determined by loss in weight on drying and density by flotation in bromobenzene-xylene. The orthorhombic unit cell dimensions were determined by X-ray defraction, the best patterns being obtained from wet crystals of 0.3 to 0.5 mm. diameter mounted in Pyrex capillary tubes in contact with mother liquor. The dimensions found were $a_0 = 69.29$, $b_0 = 70.42$ and $c_0 = 156.5$ Å, with 46.2 *per cent.* moisture and density 1.114, corresponding to a molecular weight of 35,400 (standard deviation = 400). For air-dried crystals, the corresponding figures

were 60.7, 61.0 and 112.4 Å, 9.8 *per cent.* and 1.259, giving a molecular weight of 35,600.

C. R.

Oxidation of Glucose by Yeast, Studied with Isotopic Carbon. S. WEINHOUSE, R. H. MILLINGTON and K. F. LEWIS (*J. Amer. chem. Soc.*, 1948, **70**, 3680-3683). Recent studies show that acetic acid, an intermediary of animal fat and carbohydrate metabolism, may also be intermediate in the oxidation of carbohydrate by yeast. Evidence was sought through the oxidation of glucose, in presence of isotopic acetate (labelled in the carboxyl carbon) by Fleischmann baker's yeast and *Torulopsis utilis*. Cells of the test organisms were depleted of reserve nutrients by aeration of aqueous suspensions for 16 hours, after which the cells were shaken for 7 hours at 25° C. in presence of the substrates. Alcohol, acetate, CO_2 , succinate and citrate, cell lipids and cell residues were isolated after fermentation and examined for isotopic carbon. Dilution of the isotopic content of the acetate during fermentation occurred, the implication being that some acetate arose by oxidation of the non-isotopic glucose. Both glucose and acetate appeared to participate in the formation of succinic and citric acids, suggesting that the tricarboxylic acid cycle is a common pathway for the complete oxidation of both glucose and acetate. The absence of C^{13} from the alcohol formed implied its derivation from glucose by reactions not involving acetate nor substances in equilibrium with it. From the observed distribution of C^{13} , at least 50 *per cent.* of the completely oxidized glucose could be accounted for in all experiments as passing through the intermediate stage of acetate. The fermentation and oxidation pathways of glucose may be common to the acetaldehyde stage, diverging thereafter. The results with *T. utilis* showed that the formation of acetate from glucose was not confined to highly fermentative species. It is plausible that the small amounts of C^{13} appearing in cell components arose by way of the tricarboxylic acid cycle in a manner comparable to the formation of glycogen in animal tissues.

The occurrence of CO_2 assimilation was tested by yeast fermentations of glucose in the presence of isotopic bicarbonate. Whilst appreciable assimilation of $C^{13}O_2$ was observed, especially under anaerobic conditions, it is of minor importance in succinate formation. All the isotope appeared as succinate carboxyl.

C. R.

REVIEWS

Refrigeration Note Book. By A. E. MILLER, M.Inst.R., M.S.E. Pp. xii + 328 with 42 illustrations. London: Leonard Hill, Ltd., 1948. Price 10s.

In writing this small volume, approximately 5×3 inches, the author has endeavoured to produce a compendium of information, based upon his long experience in the refrigeration industry, to which reference may be made by anyone at any time during the progress of his work. Owing to its size the book may be carried in a small pocket but, notwithstanding this, it contains a mass of valuable information on the practical aspects of most branches of the industry, this being supplemented by a series of 67 tables providing a variety of data.

In the preface the author emphasizes the necessity for conciseness in the presentation of the subject matter, but in spite of this he has introduced several items which are redundant. As examples we find that the specific heats given on p. 7 are nearly all repeated in Tables 11 and 12; over three-quarters of the data on the pressure of gases in Table 14 are again included in Tables 15, 16 and 17, and several of the tables might have been curtailed by the omission of data referring to the rarer metals, various organic substances, sulphur, mud, carbon monoxide and other materials of no importance in refrigeration. Some inconsistency is shown in the extent of Tables 15, 16 and 17 for whilst that dealing with the properties of ammonia extends over ten pages the corresponding details for carbon dioxide and sulphur dioxide are each limited to one page.

It is greatly to be regretted that the excellent qualities of this work as a whole are marred by several more or less serious errors which might have been avoided by careful proof correction. Thus on page 5 we are told "At this temperature (63 to 64° F.) water is at its maximum density" but the true temperature for this is 39° F. The specific gravity of oak is stated to be 1.2 on page 221, but this is inconsistent with the fact that the wood floats upon water. Page 168 on which the chemical tests for impurities in water are described, provides several inaccuracies not only in spelling but also in some of the deductions from the tests given. Two contradictory statements are found on pages 129 and 211, the first being "A good insulating material must have excellent thermal conductivity," and the second referring to the Baudelot cooler states: "With the wort flowing from the top to the bottom on the outside and the cooling medium from the top to the bottom full advantage can be taken of the counter current principle."

Several items in the index are liable to be overlooked, since they are inserted under the wrong letter and are not cross-indexed. Thus we find "Construction of thermometer," "Example of volumetric efficiency," "Other forms of cooling," "Required thickness of cork," etc. Obviously these should have been inserted according to the principal words: Thermometer, Volumetric, Cooling and Cork.

In view of the above unfortunate defects, and of others not mentioned, it is evident that a thorough revision of the text is necessary before full reliance can be placed upon the information provided. T. J. WARD.

Copper Compounds in Agriculture. London: The Copper Development Association, Radlett, Herts. 1948. Pp. 117 + 46 illustrations.

That compounds of copper are important in Agriculture and Horticulture is well known, but few who read this book will fail to be impressed by their great diversity of uses and the large annual requirements for crop protection alone. Without doubt, Bordeaux mixture has contributed more to the world's food supply than any other material used for crop protection. That the Irish Famines of 1845 and later years, solely due to the ravages of potato blight, would never have occurred had this fungicide been available, is only one example of the importance of copper to agriculture. The author and publishers of this book are to be congratulated on producing a well-balanced survey, easy to read and full of interest. It is not designed for the research worker; treatment is simple and comparatively few references to published research are quoted, though there is a useful Bibliography for further reading.

The first chapter of the book deals with fungi, their growth and reproduction and conditions which influence the establishment of pathogenic fungi on plant tissue. Then follows an account of the various copper preparations used as fungicides. Although many copper fungicides have become available since Bordeaux mixture was discovered in 1885, we find that this material, in spite of certain disadvantages, still retains its wide popularity, not only because of its high fungicidal value and cheapness, but because of its marked adhesive properties. In this latter connection a point not made is that the excess lime in the spray carbonates as the deposit dries, thus "mortaring" the fungicide to the leaf. Following a chapter on Spraying and Dusting Equipment, we have the chief section of the book which is

devoted to specific diseases of crops and their control. Although most of the diseases dealt with can be attacked successfully with copper compounds, in cases where other fungicides find applications, as for instance lime sulphur for apple and pear scabs, this has been stated. Similarly, in the section on Seed Treatment, it is pointed out that organic mercurials have superseded copper compounds for the control of seed-borne cereal diseases. A description of hop downy mildew disease and its control by Bordeaux mixture spraying worked out by Professor Salmon, is given. There are also three excellent photographs illustrating diseased leaves, shoots and cones.

The chapter on Copper Insecticides deals mainly with Paris Green and copper sulphate, the former substance still being widely used in crop protection, for the destruction of mosquito larvae and in poison baits. It is interesting to read in the next chapter, on Weed Control, that in spite of the recent developments in this field, copper chloride is still the herbicide of choice against certain weeds in cereals.

The importance of copper, a vital "trace" element, in plant and animal nutrition is now well established, and the following chapter gives an account of deficiency diseases arising through lack of the element. The rest of the book gives an account of copper as an antiseptic and its use in industrial microbiology, including the rot-proofing of textiles and timber and water treatment.

The author has undoubtedly succeeded in producing a volume which deserves a place on the bookshelf of all agriculturists. In bringing together in a clear and concise manner the various uses of copper in agriculture it provides useful data, gives a concise account of the diseases and problems which can be attacked and, wherever possible, details of the treatment to be adopted. The book is printed on excellent quality paper and there are 46 well reproduced photographic plates. It is supplied free of charge on request to those giving evidence of their genuine interest.

R. L. WAIN.

Cours de Brasserie, Vol. I. By JEAN DE CLERCK. The University, 32 rue de Diest, Louvain. 1948. Price 450 Belgian francs.

The appearance of a new text book on brewing is a major event in the industry; Professor de Clerck's work is certain of a great welcome throughout the brewing world. It is indeed particularly appropriate that the new work should come from Belgium, the country with the greatest beer-consuming population in the world; its authority is increased by coming from the Catholic University of Louvain, where the brewing school established as long ago as 1887 now grants a Diploma in brewing of the status of a University degree, demanding

a five-year course from its students. *The Ingénieur Chimiste et des Industries Agricoles* who has sat at the feet of Professor de Clerck may well be satisfied that every significant aspect of the science and practice of brewing has been deployed for his examination.

This "vast and magnificent work" as Professor Verhelst justifiably describes it in the introduction is by no means exclusively academic. The author not only deals comprehensively with the varieties and composition of the raw materials and with the function and underlying theory of each stage of the brewing process, but includes innumerable diagrams of the principal types of plant used and advises the brewer in unequivocal terms as to the best means of achieving his results, with a steady warning throughout of the un wisdom of sacrificing quality to commercial expediency.

The work will be in two volumes. The first and present volume deals with the materials and processes of malting and brewing; the second, to appear shortly, will deal with analysis and scientific control. Of the 600 pages of the present volume, 90 deal with raw materials, 170 with malting, 120 with brewing in its restricted and probably proper sense of the brew-house operations, 150 with fermentation, storage, racking and bottling, and the last 60 with the composition, quality and faults of the finished product.

Though the author naturally treats continental methods in greater detail and with greater knowledge, his survey of brewery methods is world wide. While some of his facts dealing with raw materials and gravities are necessarily those of pre-1939, his summary of theory is very up-to-date and includes references to researches published in 1947. There is a useful account of pre-war production of barley throughout the world, the palm for the world's best brewing barley of those days being awarded to the Hanna barleys of Moravia. The author reminds us that such names denote a district and not a variety, and puts in a plea for commercial guarantees of variety. In spite of his recommendation that the nitrogenous content of barleys should be expressed as nitrogen, he conforms largely to the obsolescent and inaccurate convention of multiplying the nitrogen figure by 6.25 and calling it protein. The protein figures he gives for the best barleys are higher than would be considered desirable in this country. He warns the student that there is no known method of physical or chemical examination of barley which will tell us with any certainty that the barley will make good beer and he puts in a plea for the systematic trial of individual varieties as to their suitability for different beers. He perpetuates the discredited theory that excess of nitrogen in the barley or malt leads to bacterial

instability in the beer and generally does not appear to be so familiar with recent bacteriological research as he is with chemical theory.

The common practice on the Continent of low temperature fermentation, storage and service, empirically developed no doubt to give the brewer greater control over his product in continental temperatures, has naturally resulted in more attention being paid to protein stability than to biological stability. This is reflected in the continental evaluation of hops by the bittering value as distinguished from our preservative value; for this the author

prefers Wöllmer's formula $\alpha + \frac{\beta}{9}$ rather than

the former British $\alpha + \frac{\beta}{3}$. The present British fashion of using only the alpha resin as a measure of hop value is not referred to, but it is interesting to note that this practice brings our values nearer to the continental, though by a different route. What is certain is that neither formula tells the whole story.

Malting is treated at great length and in great detail from barley drying to evaluation of malt, with many diagrams of plant. All methods are considered; for large installations the author favours pneumatic systems which he claims are as controllable as floor malting; the whole section is of great interest to-day. Brewing proper, or the preparation of the hopped extract, is treated chiefly according to continental practice and the infusion mash tun gets little attention. This is as would be expected and the English reader will not go to the book for instruction in his own methods. Top fermentations are dealt with a little more fully, and the discussion of conditioning in storage tank and in bottle may well be studied by those who still wish the flavour of a matured ale to be something more than a nostalgic sentiment. The author, in common with most continental observers, gives too much credit to the secondary yeast for the characteristic flavour of British beer; *Brettanomyces* if it ever was a significant agent of after-fermentation is to-day a museum piece.

These, however, are minor criticisms which only emphasize the width of survey of the Professor's book; the extent of detail is amazing. We have at last two complementary and authoritative text-books based on fact and free from mystery, Lloyd Hind's which, alas, will never be completed, and the present work in French; these authors are possibly the last two to attempt a comprehensive and majestic survey of the whole field of brewing. Advancing

knowledge will demand specialized monographs on each aspect.

The French of the present work is admirably simple, or your reviewer, who started to skim the cream, would not have stopped to read every word. A most stimulating work which no brewer who studies his subject can do without.

B. M. BROWN.

British Chemicals and their Manufacturers.

Pp. 141. London: Association of British Chemical Manufacturers. 1949.

Some of our readers will be familiar with former issues of this valuable production and the present edition supercedes that published in 1946. It comprises an up-to-date directory of the principal British manufacturers of chemical products and the first section provides a grouping of all varieties of chemicals under seven headings, and a list of firms with their respective group-headings according to the class of materials supplied by each. This is followed by a directory of members of the association together with code numbers, addresses and other trade details and a similar list of affiliated associations. The next 67 pages contain an extensive list of chemicals of all kinds, each with the code numbers of the firms from which it may be obtained. This is used in conjunction with a loose card bearing the code numbers of firms arranged in numerical and alphabetical order.

A feature of considerable utility is provided in an alphabetical list of nearly 2,000 proprietary and trade names of chemical products together with either their composition or uses and the names of the manufacturers. The origin of many of these trade designations is obvious, but to determine the sources of others would be an interesting speculation. The book concludes with a series of proprietary and trade marks together with the names of the firms to which they pertain.

From the above description it will be evident that this compilation is of considerable value to those who buy chemicals of any description, and will save much time when ordering. The text has been carefully prepared for a fairly detailed test has failed to detect an error of any kind; the typography is easily legible throughout and the volume is well bound in cloth boards. This thoroughly reliable directory will be supplied free of charge by the publishers at 166, Piccadilly, London, W.1, to anyone who will provide a genuine indication that he is likely, as a purchaser of chemicals, to make good use of it.

T. J. WARD.

JOURNAL OF THE INSTITUTE OF BREWING

MONTHLY REVIEW

FOOD

IN the issues for January and February, 1949, *Analytical Chemistry* has published its first annual review of developments in fundamental analysis and the application of these developments in various industries. The articles comprising the review are written by different authors, as in the *Annual Reports* with which many of us are familiar in this country, and one which will have particular interest for readers of this *Journal* is entitled "Food," and is contributed by B. L. Oser (February, pages 216-227).

The review covers the published work of the last five years. During this time, says Mr. Oser, the major advances in food analysis have come from the application of spectrophotometric, chromatographic and microbiological techniques, and much of the work done by these new methods has been on the determination of vitamins, trace minerals and amino acids. The methods are grouped under the headings of Moisture, Carbohydrates, Proteins and Amino Acids, Vitamins, Inorganic Elements, Disinfectants, Preservatives and Insecticides, and—since the food chemist is concerned not only with establishing the identity, purity and nutritive value of foods, but also the conditions causing their spoilage—there is a section on Decomposition and Contamination. Methods which do not fall under any of these headings are placed in a final Miscellaneous section.

Some idea of the scope of the work is given by the fact that there are references to as many as 395 publications, and it will be obvious that with such an extensive field to survey the author can seldom do more than refer to a piece of work, and such comments as he is able to make are necessarily brief. Some of the chromatographic and microbiological methods of separating and assaying amino acids that are mentioned, and the physical and microbiological methods for vitamins, are familiar tools in the hands of

research workers in the brewing industry. In regard to vitamins, Mr. Oser stresses the continued importance of the biological assay, since we cannot be sure that extraction methods and chemical reactions will always give a reliable measure of the physiologically available vitamin content of animal and plant materials.

If the author has had to use words sparingly, he has used them to good purpose, and our reading of the review leaves an almost overwhelmingly clear picture of the range and variety of problems associated with food which are receiving attention from analysts.

* * * *

SAMPLING

The *Proceedings* of the American Society of Brewing Chemists for 1948 contains a report of the work carried out during the year of a Sampling Committee which was set up to determine whether sampling methods are adequate for the various methods of analysis required in quality control tests. The preliminary investigation made during the past year has applied statistical methods to the problems involved in sampling large lots for analytical purposes, such as, for example, the sampling of consignments of barley and malts. The major problem of sampling, namely that of obtaining a sample of sufficient size to be representative of the whole, is discussed against a background of the statistical Law of Distribution. The solution of this problem lies in the study of the amount and range of variations in the lot, the effect of distribution becoming less important as these quantities decrease. A study of the distribution of variants in laboratory size samples has revealed that there is a need to review present sampling procedures for grain analysis.

The problem of obtaining a random sample is discussed with respect to the effect of segregation and periodicity. In addition,

this problem raises questions of personnel; it is essential that a sampler should be trained to understand his job.

The Committee consider that some of the sampling methods in use are inadequate and recommend that steps be taken to study gross sampling of large lots and the progressive quartering of samples to laboratory size; that data should be obtained to calculate the necessary sample size; and that, when appropriate, statements of the statistical accuracy of the method should be included in analytical procedures. It is also proposed to make a study of available sampling equipment and methods with which the Brewing Industry is concerned, so that improvements can be made. For the future, the Committee propose to devote itself to the collection of data and the development therefrom of methods of sampling suitable for general use in the Brewing Industry.

* * * *

STATISTICS

The Statistics Committee of the American Society of Brewing Chemists has been occupied with three suggestions. The suggested compilation of a glossary of statistical terms for inclusion in the A.S.B.C. *Book of Methods* is regarded as being unsatisfactory without specific examples by way of illustration. Familiarity with statistical terminology can best be accomplished, it is believed, through the medium of textbooks in which statistical methods are applied to subjects in the malting and brewing fields. Regarding the proposed revision of the section on sampling in the *Book of Methods*, the Committee think it unwise to complicate the present general procedure by the introduction of special procedures. At the same time they recognize that serious errors in certain determinations do occur, but point out that sampling is only the means to the analytical end and the analysis must be considered in designing the best methods of sampling.

The application of statistical procedures to the data obtained by the Committees of the A.S.B.C. has not yet been undertaken, but it is considered desirable that, before this work is started, the application of correct procedures should be considered. In addition, it is strongly recommended that the plans of committees undertaking studies for the Society should first be referred to the Statistical Committee.

Two simple statistical procedures are appended by way of illustration of their application in laboratory work, namely, the calculation of standard deviation, and the comparison of mean values.

* * * *

INDUSTRIAL APPLICATIONS OF RADIONUCLIDES

This is an unusual headline to find in a journal devoted to the science and technology of fermentation. What, for example, is a radionuclide? It is an element whose atoms are radioactive. They may be naturally so, as in radium, or their radioactivity may be artificially produced as in that monument to nuclear fission, the atomic pile. It would seem to be a far cry from radionuclides to methods of analysis, but the relations between the two are explained in a most interesting paper written by J. W. Irvine, jun., and appearing in the March, 1949, issue of *Analytical Chemistry* (pages 364-368). This paper was one of seven contributed to a Symposium on *Nucleonics and Analytical Chemistry* which was held in the United States last summer, and in it Mr. Irvine describes how radionuclides are being used as tracers and as sources of radiation in the solution of analytical problems. A tracer is an element whose movements can be followed by reason of its radioactivity, whether it is the partner in a series of chemical changes or merely the disinterested associate of important, but less easily detected, substances. An analogy exists in the use of fluorescein to follow the movement of underground water. To pick one of a number of industrial uses of tracers as an example, a knowledge was needed of the amount of ink laid down in printing. The information was gained by treating the varnish used in the ink with a compound of phosphorus containing a small amount of the radioactive element, and measuring the intensity of the radiations given off by the print.

In tracer work the emphasis is on the material emitting the radiation, and the radiation itself is used only for measurement. If a radionuclide is used as a source of radiation this emphasis is reversed; the material is of relatively small importance compared with the radiation. The radiation may owe its importance to its effect on matter, as in preventing the accumulation of static

electricity by ionizing the adjacent air, or its value may lie in the effect which matter exerts upon it, as in gauging the thickness of an absorbent film from the amount of radiation which the film cuts off when placed between a radioactive source and its detector. To quote once more an industrial application, the level of liquid in a closed tank can be indicated and automatically recorded by having a small source of radiation on a float and a detector in the roof of the vessel. This is because the intensity of radiation from a point source changes as the inverse square of the distance between the source and the detector.

When it comes to translating these methods from the small scale of the laboratory to the large scale of industrial process control, Mr. Irvine gives the warning that unforeseen difficulties may arise. The amount of radioactivity involved may quickly reach a level at which it is a potential danger to the operatives. The problem of disposal of the radioactive material may become serious, and any contamination of the product must be avoided, not only because of the trouble this might cause to such things as measuring instruments and photographic plates and films, but also because public knowledge that the product was radioactive would have an undesirable psychological effect.

* * * *

CONTRIBUTIONS OF POLYMER CHEMISTRY TO THE PLASTICS INDUSTRY

Everyone nowadays is familiar with articles of all kinds constructed of plastic materials, both in the home, and in commerce and industry, but relatively few are aware of the phenomenal development in their manufacture during the last 25 years. A concise and interesting account of the main branches of this extensive industry has been written by C. S. Fuller and published in *Industrial and Engineering Chemistry* for February, 1949, (page 259) from which we learn that over 13 million tons of plastic materials are produced annually in the United States. The earliest work on these substances was carried out more than a century ago when investigations were being made on natural rubber, polystyrene, nitrocellulose and varnishes, but owing to the inadequacy of scientific methods at that time little progress was possible. The production of Bakelite about 1906 initiated

the modern advances in plastic developments and since 1924 the industry has made rapid headway. The extension of synthetic organic chemistry and the production of new types of machinery have greatly facilitated this progress, whilst competition between different manufacturers is enforcing the application of new procedures and the production of materials having still more valuable properties than those previously available. Polymer, or as it is sometimes called, plastic chemistry, is a combination of chemistry and physics, the latter being of greater importance, especially in the testing of the finished products. The underlying principles in the production of plastics involve the combination of two or more simple organic compounds to form substances with free radicals and thus capable of linking together, by a process known as polymerization, into long molecular chains. These when suitably treated—such as by stretching or heating—develop crystalline micelles which bind the mass into a more or less plastic material. However, the diversity of these materials renders it highly probable that this is only part of the story, and much more work will be necessary before the whole subject is fully elucidated.

In the past many theories have been advanced to account for the peculiar properties of plastics, but some of these have been confuted by X-ray analysis, together with other modern physical methods of examination. The behaviour of plastics in use led formerly to much difference of opinion, but this has been largely eliminated by the great increase in knowledge of the methods of manufacture and their physical properties. Variation in treatment during production often leads to wide differences in the physical characteristics of the resulting plastics, although their chemical composition may be identical. These products of such wide utility are a tribute to the valuable work of numerous chemists and physicists in many complex and difficult investigations, and well illustrate the value of applied scientific method in industry.

* * * *

ST. ANDREWS SYMPOSIUM

The full programme for the Symposium entitled "Recent Advances in the Fermentation Industries" has now been announced (*cf.* this *Journ.*, 1949, 59), and it is clear that the opportunity is being offered for obtaining

a most useful refresher course not only in relation to brewing problems but also in such subjects as bread making, bacterial and fungal fermentations, and the microbiological assay of vitamins and amino-acids. Even the topics not directly related to brewing are such as may well prove stimulating to brewers and brewers' chemists. A limited amount of accommodation is still available, and forms of application—which should be completed immediately—are available from the Assistant Secretary, Royal Institute of Chemistry, 30, Russell Square, London, W.C.1. Attendance at the Symposium is not restricted to members of the Royal Institute.

* * * *

THE SECTIONS

LONDON SECTION

A MEETING of the London Section was held at the Horse Shoe Hotel, Tottenham Court Road, London, W.1, on Monday, 14th February, at 6 p.m. Mr. B. M. Brown, Chairman of the Section, presided, when a paper entitled "An Outline of the Brewing Industry in New Zealand," was read by Mr. R. K. Logan, M.A. (*cf.* this *Journ.*, 1948, 293).

At the conclusion of the lecture a film which had been prepared by the New Zealand Government Department of Agriculture was exhibited, giving a description of farming conditions in barley-growing districts and showing that some very good quality two-row barleys could be produced.

In the discussion which followed, members remarked on the stability of the draught beers which were produced and compared New Zealand practice with English brewing methods.

A meeting of the Section was held at the Horse Shoe Hotel, Tottenham Court Road, W.1, on Monday, 28th March, at 6 p.m. Mr. B. M. Brown, Chairman of the Section, presided and a paper entitled "Malt Analysis; a discussion of the recent revisions in the Standard Methods of the Institute," was read by Mr. A. A. D. Comrie, B.Sc., F.R.I.C.

Mr. Comrie discussed the various determinations in the Standard Methods with particular emphasis on the effect of recent changes in the prescribed technique. A feature of the paper was the introduction of notes on incidental experiences of the author in the course of malt analyses. A new design

of moisture oven was suggested, and the use of malt extract syrup for the determination of extract in coloured malts and flakes was recommended. In conclusion Mr. Comrie emphasized the need for collaborative tests with delineation of the limits of error in the routine estimations, and pleaded for experimental study of the analysis methods in order to ascertain those features which were of critical importance so that methods might, if possible, be simplified without loss of accuracy.

A general discussion followed the reading of the paper, in the course of which English and Continental methods of malt analysis were compared and certain alternative procedures in standard methods were considered.

A meeting of the Section was held at the Horse Shoe Hotel, Tottenham Court Road, W.1, on Monday, 11th April at 6 p.m. Mr. B. M. Brown presided and a paper entitled "Cooperage Comments" was read by Mr. W. Lindley Wilson, who described the method of cleaving timber to produce cask staves and discussed the supplies of wood now available for cask-making. After referring to the use of various machines to replace the traditional hand labour of the cooper, the lecturer emphasized the need for correct seasoning of a cask before bringing it into use and in conclusion discussed the desirability of lining with enamel or pitch.

In the general discussion which followed, several members expressed the need for standard specification of cask measurements.

SCOTTISH SECTION

THE 28th Annual Dinner of the Section was held in the North British Hotel, Edinburgh, on 18th March, the Section Chairman—Mr. L. Fletcher—presiding over an attendance of 150. The guests included Bailie W. D. Gerrard (City of Edinburgh), Prof. Sir Sidney Smith (University of Edinburgh), Lt.-Col. F. E. Tetley (Chairman, the Brewers' Society), Mr. M. V. Courage (President of the Institute), Mr. Sanders Watney (Hon. Treasurer of the Institute), Col. W. H. Whitbread (Chairman, Research Committee), and Sir Robert Nimmo (President, Brewers' Association of Scotland). The Toast "The Institute of Brewing and the Scottish Section," was proposed by Sir Sidney Smith, reply being made by Mr. Courage and Mr. Fletcher; Dr. I. A. Preece proposed

"Our Guests," for whom Lt.-Col. Tetley responded. The proceedings concluded with the proposal by Mr. A. Clark Doull of the Toast "The Chairman."

Associated with this function was an exhibition of New Varieties of hops of the 1948 growth arranged on 17th and 18th March by Messrs. Wigan, Richardson & Co., and a meeting of the Section was held on 17th March under the Chairmanship of Mr. L. Fletcher. The Hon. Secretary reported that, after meetings between brewers, distillers, and farmers a paper on recommended varieties of Scotch malting barleys had been prepared by Messrs. L. Fletcher and A. M. Slight and forwarded to the National Farmers' Union of Scotland, for publication in the *Journal* of the N.F.U. for the information of farmers.

A paper was then read by Major D. Wigan on "Mechanical Harvesting of Hops in U.S.A." After dealing with the difficulties the English grower experiences in obtaining labour, the lecturer referred to the relative advantages and disadvantages of mechanical picking, describing the Dauenhauer machine which he had studied on a visit to America and giving details of the cost of the machine and of its operation. He expressed the opinion that mechanical picking for large gardens seemed to be the solution to present labour difficulties. A colour film on mechanized picking, taken in America, was shown. Discussion followed, in which part was taken by the Chairman and by Messrs. Morris, A. Davidson, M. R. Dewar, R. Allan, and J. Speirs.

YORKSHIRE AND NORTH-EASTERN SECTION

THE Annual Banquet of the Section was held at the Queen Hotel, Harrogate, on 8th April, Mr. R. S. Sandbach, Chairman of the Section, presiding over a large attendance of members and guests. The Toast "The Institute and the Section," was proposed by Major C. York, M.P. for the Harrogate Division, who referred to difficulties encountered by brewers at the present time, making special reference to the problem of acceptable barley varieties. Mr. M. V. Courage (President of the Institute) in reply referred to the work of the Institute and to the regard in which it was held overseas, looking forward confidently to the successful development of the Research Scheme. Mr. Sandbach proposed the Toast of "The Guests," who included (in addition to

Major York and Mr. Courage) Councillor A. V. Milton (Deputy Mayor of Harrogate), Lt.-Col. F. E. Tetley (Chairman, the Brewers' Society), Mr. J. Suddaby (President, I.B.G.), Mr. J. Griffiths (General Secretary, I.B.G.), and Mr. C. E. Randell (Editor, *Brewers' Journal*), as well as representatives of Local Sections of the Institute and I.B.G. Councillor Milton and Mr. Randell replied.

THE Spring Meeting of the Golfing Society of the Section was held at the Lindrick Golf Club, near Worksop, on Friday, 13th May, 1949. The results were as follows:—

Morning—Sutcliffe Trophy (Medal): Winner, E. R. Collins; score 76 nett. Runner-up, E. B. Mather; score 78 nett.

Mr. R. B. White returned a score of 77 nett, but was not eligible to compete for the Sutcliffe Trophy.

Afternoon—Committee Prizes (Fourball—bogey): Winners, N. Garthwaite and H. St. G. Gallagher, 3 up.

Runners-up:

E. B. Mather and P. Branston;	} 2 up
E. R. Collins and S. H. Robson;	
G. Troop and H. Birkenshaw	

* * * *

OBITUARY

ROBERT VALENTINE REID

MR. ROBERT VALENTINE REID, former Chairman of Hugh Baird & Sons, Ltd., of Glasgow, London, etc., passed away on the morning of 21st April, in his 77th year. Mr. Reid joined Hugh Baird & Sons in 1902, and had been a director for 43 years—in fact, up to the time of his death. He was Chairman of the company from 1937 until the end of 1948, when he relinquished the appointment for health reasons.

The high esteem in which he was held by all with whom he came into contact, and the unselfish zeal with which he devoted himself to the interests of his associates, are reflected by his many activities and the positions which he attained. His work for the Institute of Brewing, of which he was a member for 45 years, was particularly outstanding. Thus, he was Chairman of the Scottish Section from 1914 to 1919 and when, subsequently, he went to live in London he continued to represent the Section on Council for a

number of years. During the period 1924-26 he occupied the office of President of the Institute, and his total period of service on Council amounted to no less than 36 years. In addition to his work for the Institute, Mr. Reid was Hon. Treasurer of the Maltsters' Association for a very long period and also served on the Committee of the Hop Merchants' Association; he occupied the Chair-

manship of the Malt Roasters' Association for many years and was a past Chairman of the Allied Brewery Traders' Association.

His great wisdom and wide experience will be severely missed, but those who had the privilege of knowing him will always treasure the memory of his personal charm and the value of his kindly encouragement.

J. PRIMROSE BROWN.

TWENTY-EIGHTH ANNUAL MEETING OF CORPORATE MEMBERS OF THE
 INSTITUTE OF BREWING, HELD AT THE OFFICES OF THE BREWERS'
 SOCIETY, 42, PORTMAN SQUARE, LONDON, W.1, ON WEDNESDAY,
 18TH MAY, 1949, AT 2.45 P.M.

Mr. M. V. COURAGE (President) in the Chair and 20 Members

(1) *The Minutes.*

The Minutes of the 27th Annual Meeting of the Corporate Members, held on the 25th May, 1948, were read and confirmed.

(2) *Annual Accounts.*

It was moved by Commander A. Cory-Wright, seconded by Mr. G. T. Cook, and RESOLVED I: That the Research Fund Accounts for the year ended 31st December, 1948 (this *Journ.*, 1949, pp. 94-95), be and are hereby approved and adopted.

(3) *The Report of the Council.*

It was moved by Commander A. Cory-Wright, seconded by Mr. G. T. Cook, and RESOLVED II: That the Report of the Council (*i.e.*, those paragraphs dealing with the research; this *Journ.*, 1949, pp. 80-91) for the year ended 31st December, 1948, be and are hereby approved and adopted.

At the conclusion of the meeting Sir Ian Heilbron submitted a progress report on the reconstruction of the Research organization.

FORTY-SIXTH ANNUAL GENERAL MEETING OF THE INSTITUTE OF BREWING,
 HELD AT THE OFFICES OF THE BREWERS' SOCIETY, 42, PORTMAN SQUARE,
 LONDON, W.1, ON WEDNESDAY, 18TH MAY, 1949, AT 3.15 P.M.

Mr. M. V. COURAGE (President) in the Chair and 25 Members

THE Minutes of the 45th Annual General Meeting, held at the Goring Hotel on the 25th May, 1948, and of a subsequent Special General Meeting held at the same address on the 22nd June, 1948, were read and confirmed.

ANNUAL ACCOUNTS

General Accounts.—In moving the adoption of the accounts, the HON. TREASURER (Mr. S. Watney) stated that the Income for the year showed a net increase of £1,776 as compared with 1947, whilst the Expenditure showed a net increase of £606. The General Account had thus finished the year with an excess of some £1,170 over expenditure, which had been carried to the Balance Sheet.

This result was due mainly to a more appropriate division of Office salaries as between the General Account and the Research Fund Account.

With regard to the Investments, the £1,000 Conversion Loan (3 *per cent.*), which reached its redemption date in 1948, had been sold and, in its place, some £1,950 National War Bonds (1952-54) had been purchased.

The Balance in favour of the General Account at the end of the year was about £10,000, of which about £5,900 was represented by Investments and £1,900 by cash.

Research Fund Accounts.—The balance in favour of the Research Fund Account at the end of 1948 was about £144,700, of which about £109,450 (£31,000 belonging to the old account) was represented by investments, £5,500 by cash, £16,100 by Lyttel Hall and equipment, and Sundry Debtors (less Creditors) £13,635.

Sundry Debtors amounted to £16,037 (of which £15,000 was due from the Brewers' Society) and Sundry Creditors £2,402.

The Expenditure on Research during 1948 was about £20,000, as compared with £10,000 in 1947.

The allocation of £60,000 *per annum* from the Brewers' Society was now the only income for Research, with the exception of the subscriptions from non-Brewer Corporate Members and the interest from investments (now about £2,350 *per annum*).

Altogether they had received £120,000 from the Society, that is to say, during the 2½ year period Oct. (1946)-Dec. (1948).

The expenses had been approximately as follows:—

<i>Total Capital Expenditure</i> (1947-1948)			
	£		£
Investments	78,000		
Purchase of Lyttel Hall ..	15,500		
Apparatus, etc.	600		
		94,100	
<i>Total Revenue Expenditure</i> (1947-1948)			
		30,050	
			£124,150

With regard to the immediate future, the prospective annual expenditure (other than at Lyttel Hall) was as follows:—

	£
Wye College (Hop Research Department) (Sanctioned up to March, 1950) ..	5,000
Hop Investigations, Manchester	2,000
Barley Investigations	205
Research Laboratories, Birmingham ..	6,000
Extra-mural Grants	5,000
	£18,205

This expenditure covered work carried out extra-murally whilst Lyttel Hall was being completed. Some of this work would, eventually, be merged in that of the Institute's Research Establishment (to be known as "The Brewing Industry Research Foundation") and was catered for in the expenditure of £45,000 recently authorized by the Finance Committee.

The motion for the adoption of the Accounts was seconded by Mr. L. Fletcher and carried unanimously.

ANNUAL REPORT

The President moved the adoption of the Annual Report, and referred to one or two items of the Report as follows:—

List of Members.—He was gratified to report a further increase of 151 new members during the year ended 31st December, 1948. Although this was less than last year, it

represented a total increase of 420 members during the last two years.

There were some who thought, however, that Rule "9", that was to say, the Rule which governed the admission of Ordinary Members, was somewhat too wide in its scope, and the Council had recently appointed a small Committee, consisting chiefly of the Chairmen of the various Sections, to review the position.

It was regrettable to find, however, that some 750 members of the Incorporated Brewers' Guild were still outside their ranks.

Examinations.—Some 64 candidates presented themselves for examination last year, and there would be a further increase this year. There was no doubt that the majority of firms having vacancies to fill now paid due regard to the claims of those who had, by means of the examinations, obtained a receipt for their education. In this connection, he would draw attention to the fact that arrangements could always be made for the examination of candidates abroad, and for those prospective brewers who might be temporarily in the Army, or serving in one of His Majesty's ships. An examination was being held this year in Toronto.

John S. Ford Memorial Trust.—A second award under this Trust had again gone to a Scot. He hoped that, during 1949, the award would come south of the Border!

Employment Bureau.—The Institute's Employment Bureau had been very active during the year. He would impress upon members, however, that no name was entered in the books of the Bureau, unless the applicant had obtained the sanction of his firm to look for another appointment.

Registration of Student Members.—Some 20 Students had already been registered under the new Scheme. On the one hand, it was of undoubted advantage for the Institute to be able to estimate within reasonable limits the number of brewing students in training at any one time, and thus have an opportunity of keeping in touch with them. On the other hand, it was of advantage to the students to know precisely their position in regard to the examination regulations and syllabus.

The Journal.—Although the Institute still had to limit publication to six issues *per annum*, there was a likelihood of paper supplies being adequate to immediate requirements.

Hospitality Committee.—They were now in a position to repay, to some extent, the hospitality of those Continental firms who had done so much to make the itineraries of the John S. Ford Scholars so interesting and valuable. The Scheme for the exchange of British and Continental Students was now well under way, and two or three Continental brewers had already been "adopted" by several of the firms who were collaborating in the Scheme.

European Brewery Convention.—The Congress which was to be held in Lucerne at the end of May would, he hoped, be attended by some 40 Members of the Institute of Brewing, and he had no doubt that an account of the proceedings would be published in the *Journal*. The Third Congress would, it was anticipated, be held in London in 1951.

The Sections.—He would like again to congratulate the Sections on a very active and valuable year's work. The Institute owed a very great deal to the enthusiasm of the local officers and committees.

Offices.—It might not be known to all that, although the Institute's address was still c/o The Goring Hotel, and the telephone number remained unchanged, they were compelled, at very short notice, to relinquish the rooms which they had occupied for nearly five years. However, the Hon. Treasurer came to their aid, and it gave him great pleasure to report that the Secretary and his Staff were now very adequately housed, for a time at least, in a property belonging to Messrs. Watney, Combe & Reid. This Company had almost gone out of its way to make the Institute welcome, and everybody concerned was very grateful to Mr. Sanders Watney for all that he had done in that connection.

Research.—The Institute had made great progress in its Research plans since the last Annual General Meeting, and he desired to express a very cordial welcome to Sir Ian Heilbron, whom they were so pleased to see at the meeting that afternoon.

The Motion for the adoption of the Report was seconded by Mr. B. M. BROWN and carried unanimously.

RE-ELECTION OF PRESIDENT

It was moved by Commander A. CORY-WRIGHT, seconded by Mr. J. D. DANIELL, and unanimously RESOLVED that Mr. M. V. COURAGE be and is hereby re-elected President of the Institute for the year 1949-1950.

RE-ELECTION OF HON. TREASURER

It was moved by Mr. CHRISTOPHER GEORGE, seconded by Mr. G. T. COOK, and RESOLVED that Mr. SANDERS WATNEY be and is hereby re-elected Hon. Treasurer of the Institute for the year 1949-1950.

ELECTION OF MEMBERS OF THE COUNCIL

The Secretary reported that two nominations, *viz.*, in favour of the election of Mr. A. H. Brook and Mr. R. A. Houghton, had been received from the general body of members under Bye-Law 50, and that the Council had nominated Mr. J. B. Binnie and Mr. R. W. Hill Forster to fill the remaining vacancies.

The President thereupon declared Messrs. Binnie, Brook, Hill Forster and Houghton to be duly elected as Ordinary Members of the Council.

ELECTION OF LIFE MEMBERS

It was moved by Mr. R. S. SANDBACH, seconded by Mr. JAMES STENHOUSE, and RESOLVED that the following members be and are hereby elected Life Members of the Institute under the provision of Bye-Law 14, *viz.*, Mr. G. D. CLARKSON, Mr. W. R. FARQUHAR and Mr. J. W. NEALE.

APPOINTMENT OF AUDITORS

Mr. B. BINSTED proposed, and Mr. G. C. MATTHEWS seconded, the re-appointment of Messrs. Thomas & Co., as Auditors, and the motion was duly carried.

VOTE OF THANKS TO THE CHAIRMAN

On the proposition of Mr. BINSTED a hearty Vote of Thanks was accorded to the President.

THE INSTITUTE OF BREWING ANNUAL BANQUET

THE Banquet was held at the Savoy Hotel, Strand, London, on Wednesday, 18th May, 1949, Mr. M. V. Courage, President, being in the Chair. 450 members and guests were present.

Following the loyal toasts, Lt.-Col. F. E. Tetley, D.S.O., T.D., Chairman of the Brewers' Society, said that he was very sensible of the honour accorded him of proposing the Toast of the Houses of Parliament, coupled with the name of the Rt. Hon. J. Chuter Ede, H.M. Secretary of State for Home Affairs. Mr. Chuter Ede could be claimed as a distinguished member of the confraternity, as the presiding genius over a large brewery with its satellite inns. He was a Minister whose temperate and good-humoured handling of matters concerning their trade (matters which were even now at issue) had commanded respect. Mr. Strachey was also very welcome; he might almost be claimed as an Allied Trader, for it was to him that the brewer turned for his malt and sugar, never—knowing him to be not ill-disposed toward the national beverage—entirely without a hope that rations might be increased. They were privileged also to have Lord Llewellyn present.

Two great institutions evolved by the British people were Parliament—and the "pub." Both were clearly shaped by the national character and represented abiding traits: a readiness to hear all sides and a capacity for collective deliberations. In both there was much the same respect for qualities of character, and in neither was there much enthusiasm for quibbling; most valued was practical good sense, with that leavening of humour which the Home Secretary knew so well how to dispense. Above all was the insistence, in both places, on the right to free speech. Just as the Chamber of the House of Commons was blitzed, so were many hundreds of pubs, and it was hoped that the completion of the new House would quickly be followed by the re-instatement of blitzed pubs. The traditional features of both would continue.

The Trade had been a subject of interest to Parliament as long as Parliament had existed. It always would be. Many of the questions which still concerned the Government and Parliament were questions which

arose centuries ago and which were settled satisfactorily for a time, until new needs and new circumstances brought up these problems afresh. In proposing this Toast he would like to re-affirm that the loyalty of the Trade to Parliamentary institutions was complete and unreserved, and to assure the Home Secretary that they considered it their duty, in the same way as did Parliament, to serve the public.

The Rt. Hon. James Chuter Ede, M.P., assured Col. Tetley that he valued greatly what had been said. True, he was a member of the confraternity but, unlike other members, he had no intention of expanding his business as a brewer. One of his predecessors amalgamated five breweries and left him with one; but while other brewers were prepared to supply his houses wherever in the country they might grow up, he would be quite prepared to accept their beer, and would hope to distribute his favours with due regard to the wishes of the community and the availability of supplies. He greatly relished the comparison between the Houses of Parliament and the licensed houses; it had not always been made so flatteringly to Parliament.

The Toast was one that they were all entitled to regard with pride, for in the past 600 to 700 years the genius of the British—particularly the English—people had evolved a method for preserving order, retaining individual liberty at the same time; to ensure that order and individual liberty could flourish together was the supreme task of a freely elected Parliament. He believed that the strength of Parliamentary democracy would enable us to preserve, bring up to date, and even expand the conception which had slowly formed during the centuries.

He had recently been asked to arrange that permitted hours in London should be made uniform; this had been achieved in three weeks; dictatorships could not have done it much quicker! He had also dealt with the problem of the early hours of the morning; although everyone who had been to a night-club agreed it was a most boring place, the demand remained at least constant. He had made an attempt to deal with the problem in what he hoped was a commonsense way.

It was part of his duty to see that if a person wanted to enjoy himself without interfering with the reasonable enjoyment of his neighbours, he should have the opportunity of doing so.

Sir Charles Darwin, K.B.E., M.C., F.R.S., submitting the Toast of "Research," declared that research must be a co-operative business. During the past three or four years there had been a great deal of discussion concerning research, and they could all endorse its very great importance in every way. He confessed that he had sometimes felt that certain of its advocates had seemed to overstate their case; they had represented it on the basis of a lottery in which one was eventually bound to gain first prize. It certainly was a speculative business. They got some good speculation, some moderate speculation, and—sometimes—bad speculation; but in the long run they would get a handsome dividend. What was research? If they felt that what they had at the present time could not be improved on in any way he would say: "Nevertheless, you cannot afford not to research." To research was a speculation; not to research was very much more than a speculation. Was it not contrary to experience that the world should go on as it is to-day? Therefore no one could afford not to make himself ready to meet changes, and that could only be done by study and research. If anyone said: "We cannot afford research" then his answer would be: "That is just too bad." He was quite certain that they could not afford *not* to research.

Sir Ian Heilbron, D.S.O., F.R.S., President of the Chemical Society and Director of the Brewing Industry Research Foundation, said that if anyone still doubted the value of research and its immense practical applicability, they would surely have been convinced by a visit to the British Industries Fair where, to quote only one example, there was a bewildering array of plastic articles extending into almost every avenue of civilized life. All these had sprung from research, which only a decade ago had not emerged from the academic phase. The Brewing Industry was one which hitherto had developed largely against a background of traditional experience. It had reached a very high state of perfection, but there was a surprising absence of fundamental knowledge; this must be remedied if the Industry were

to reach its full stature. He would suggest that this implied more than providing a traditional British beverage. He had in mind the amazing expansion of the Fermentation Industry in the production of antibiotics such as penicillin and streptomycin, of vitamins, and of many other complex compounds elaborated in the living cell during fermentation. In launching the present scheme, the Brewing Industry showed faith and vision. From now onwards it ranged itself alongside other great industries in its determination to carry out co-ordinated research for its corporate good. He could say, as President of the Chemical Society, that the chemical community viewed this development as a happy omen, and one fraught with glowing possibilities.

In assuming the Directorship of the Research Foundation and in planning its detail, he would again emphasize that he quite failed to perceive any real conflict between fundamental and applied research, envisaging the Foundation as the scientific nerve centre for a great industry. It would, on the one hand, teach the application of such fundamental knowledge as was already available. On the other hand, by its own efforts, by supporting extra-mural work, by scientific intercourse, and by every reasonable means, it would add to that fundamental knowledge which, in an empirical art like brewing, was at present so scanty but which, just because of this empiricism, was so much more necessary. Looking further ahead, there might be envisaged a brewing centre of University status, from which would flow a supply of trained biologists both for the industry itself and for ancillary organizations. At the same time he appreciated that there existed many problems which could be solved on the basis of existing knowledge. Here, the provision of applied laboratories equipped to deal with analytical, biological, and other matters (including an experimental brewery), and forming an integral part—both geographically and in principle—of the central research institute, was in the forefront of his plans.

Major advances could not be hoped for unless there existed real knowledge of all the materials and processes concerned. Accordingly, guided by the principles laid down in the Institute of Brewing's Research Scheme, he had already thought fit to spread the net widely, and had been fortunate enough to

enlist the extra-mural interest of many of the foremost authorities in the Universities in the fundamental sciences surrounding brewing chemistry in all its many aspects—biochemistry, chemical microbiology, bacteriology, genetics, etc. It would, he suggested, be wise to see that these connections grow and mature.

On the more domestic side, building plans for Lyttel Hall at Nutfield, Surrey, were substantially complete, providing for the conversion and extension of the main buildings and the provision of a range of engineering and other workshops. He envisaged a Foundation unique in these islands, if not in the world, equipped with the finest apparatus available so that any problem in its field, whether pertaining to hops, barley or malt, yeast, moulds or bacteria, or whether it were analytical or of a process nature, could be engaged. Plans were in the hands of the architects, engineers and builders, and there was every reason to believe that it would be possible to take possession in just over a year's time. While dealing with the question of building, he would like to take the opportunity of thanking the Ministry of Food and the Ministry of Works for the help they were giving in various directions. Of the specialist scientific staff, which would number about 25 at the beginning (though the laboratories would be adequate for a larger number), some 18 members had been appointed or were in course of appointment, some already working extra-murally in the Universities. He felt that he could assert that a Research Foundation was arising which in every way would be commensurate with the importance of the Industry.

Finally, he would point out that Lyttel Hall was near enough to London to be easily visited by car, bus or train. He earnestly hoped that, once they were established, members of the Brewers' Society and the Institute of Brewing would pay many visits. "Remember," continued Sir Ian, "it is your Research Foundation and the more I can interest you in what we are doing to make you feel that you are part and parcel of it, the more certain it is that it will prosper." He would, at the same time, like to extend a cordial invitation to Ministers of H.M. Government and others who, by their presence that evening, showed their interest in the Industry.

The Rt. Hon. John Strachey, M.P., Minister of Food, proposing "The Institute of Brewing," declared that in the Food Ministry they regarded as having the greatest national importance the scientific institution at Lyttel Hall, devoted so largely to research under the auspices of the Institute of Brewing. In providing those great research facilities they were performing a great service to the community, especially with regard to their investigation into the scientific processes of fermentation. In the fundamentals of food production itself, mankind had so far advanced very little since the Neolithic Period, but biochemists were now on the verge of taking them into a new stage of food production and food preparation. He hoped they might produce, scientifically, those things such as good beer which had been produced empirically and with the accumulated craft of ages. He would only pray, in presence of those scientists, that they would see that, while there were great benefits in producing more hygienically and more efficiently, they did not lose in the production of beer its excellent taste.

The President of the Institute of Brewing (Mr. M. V. Courage) in replying to the Toast, said that he was very proud to welcome such a large and distinguished gathering of members and guests. He must not presume to trespass on the Toast which was shortly to be proposed by Col. Whitbread, but he would like to say how pleased he was to welcome Dr. Kreiss (President) and Dr. Mendlik (Secretary) of the European Brewery Convention, in which Great Britain was represented by the Institute of Brewing. The other member countries were Denmark, France, the Netherlands, Norway, Sweden and Switzerland. The Convention aimed to co-ordinate scientific work in the technical domain of malting and brewing. It sought to attain that object by means of biennial international congresses, so designed as to provide opportunities for the exchange of views on definite subjects. The first Congress was held in Holland in 1947. The second, at which the Institute would be well represented was to be held in Lucerne at the end of May, 1949. It was hoped to hold the third Congress in London in 1951, when they would do their best to repay the hospitality of their continental friends.

During the current year they had made

arrangements for the interchange of British and continental students of brewing. A number of well-known breweries were collaborating in the scheme, and he was happy to report that several continental students had already been adopted by English breweries. On the other hand, the two John S. Ford scholars, selected by the Institute on the results of examinations in 1947 and 1948, had elected to visit the breweries of Scandinavia, and he would like to take the opportunity to express thanks to the firms who had so kindly provided facilities for study and hospitality.

The Institute set considerable store by the benefits to be obtained from the interchange of opinions. The reading of papers and the sharing of discussions had been a domestic feature of their organization since it was formed some 63 years ago. Such papers as were selected for publication appeared in their *Journal*, which undoubtedly had now a world-wide reputation.

At the last dinner, in 1947, he had referred to plans for instituting a central research station in the neighbourhood of London, and they had heard from Sir Ian Heilbron of the progress made. They hoped that the Brewing Industry Research Foundation would prosper under Sir Ian's inspiring direction, and they were looking forward to the opening day, perhaps a year hence, when many who were there that night would, he hoped, grace the ceremony with their presence. The whole brewing industry was behind the venture, and they looked with confidence to the unfailing support and interest of their many distinguished guests.

Another side of the Institute's work was the bringing into the Trade of young men who aspired to become professional operative

brewers. Its work on the examination side was being increasingly appreciated by the more progressive brewers. To-day, the art of making beer was becoming more and more complicated, and it was becoming more difficult to please the public. With the Minister of Food present he thought it was right and proper to urge him to do something in the direction of allowing them to go back to the making of a reasonable national beverage. He said that, both as a brewer and as President of the Institute. They might set up model breweries to experiment with high gravities, but they must also be allowed to brew these gravities in bulk once again, and so prove to the whole world that Britain still led in the industry of brewing beer.

The Institute was going from strength to strength. They were recognized throughout the world as the authority on the technical side of the fermentation industries, and it was gratifying to find how much the Institute was appreciated in other countries. He expressed thanks to Mr. W. H. Bird, the Secretary, who had a limited staff and had lately been bandied about from office to office; he would congratulate him on his efforts during the past year and for many years before that.

Col. W. H. Whitbread proposed the Toast of "The Guests," and the Rt. Hon. Lord Llewellyn, P.C., C.B.E., M.C., T.D., in reply said he was mindful of the very helpful attitude they all adopted towards him when he was Minister of Food and later, when as a Gunner Officer whose troops were moved suddenly from South Coastal areas to the East Coast, the supply of beer to the gunners was quickly reorganized; the co-operation of the brewing industry helped them to carry on with their job.

THE INSTITUTE OF BREWING RESEARCH SCHEME

GIANT COLONIES OF *ACETOBACTER* SPECIES AS AN AID TO IDENTIFICATION

By DORA KULKA, Ph.D., J. M. PRESTON, B.Sc., F.R.I.C. and T. K. WALKER, Ph.D., D.Sc., F.R.I.C.

(Received by the Secretary: 18/12/47)

In a previous communication by Walker and Tosic (*J. Soc. chem. Ind.*, 1946, 65, 104, 180) descriptions were given of the growth, in the form of streak cultures, of various acetic acid bacteria, such cultures being prepared as diagnostic tests when examining the organisms. It has since been found that when the different species are cultivated as giant colonies on the surface of semi-solid wort-agar some develop typical forms of growth which strikingly differentiate them from others. This is a distinct improvement on the streak culture as an aid to differentiation.

Further, it has been found that differences in appearance of giant colonies of *Acetobacter* species are revealed most effectively, in some cases, if observations of the colonies are made with "dark field illumination," and photographs which prove useful as aids to identification can be taken in such conditions. In other cases photographs, equally useful for diagnostic purposes, can be taken with top lighting. It has been observed also that in the cases of motile species (excepting the cases of those pellicle-forming types* which show motility) photographs of the colonies taken with dark field illumination show clearly the zone of growth which develops round the colony of a motile species on a semi-solid medium. Hence, the method is useful as a means of detecting motility, for by microscopical examination alone it is not always possible to decide with certainty whether or not a given species can display this behaviour. Some of the pellicle-forming species show motility in hanging-drop preparations but not when grown as giant colonies on semi-solid agar, on the surface of which they develop as coherent colonies without zones.

INTRODUCTION

The usual method of detecting motility in bacteria is to look for it in a hanging-drop under the microscope, the hanging-drop being taken from a young culture in a suitable liquid medium. Tittsler and Sandholzer (*J. Bact.*, 1936, 31, 575) improved upon this by cultivating bacteria as stab-cultures in semi-solid media. When this is done the motile cells move away from the point at which they originate and thus an outer zone of diffused growth develops and becomes visible to the naked eye. Walker and Tosic (*loc. cit.*) found the latter method useful when examining *Acetobacter* species. Janke (*Z. Bakt.*,

1916, II, 45, 27) cultivated *Acetobacter* species as giant colonies on a solid medium of normal consistency and noted that some species gave forms of growth which were highly characteristic and therefore constituted an aid to identification. The present authors have found that Janke's technique can be improved considerably by carrying it out with a semi-solid medium. The usefulness of this technique is thereby increased, for when motile cells are present they are revealed clearly, in the majority of cases, whereas in Janke's solid medium motility is not observed.

As compared with the method of Tittsler and Sandholzer, which also involves the use of a semi-solid medium for stab-cultures, cultivation on a semi-solid medium in

* One pellicle-forming species, *A. pasteurianum*, forms a giant colony which shows a zone.

petri-dishes offers more favourable conditions for the growth of *Acetobacter* species, which are all more or less pronouncedly aerophilic.

When giant colonies of *Acetobacter* species were first cultivated on plates containing a suitable semi-solid medium, the authors noted that in the case of motile types there could be discerned, around the main area of growth, a zone of what appeared to be faint secondary growth. At first it was thought possible that this appearance might be produced by chemical changes caused in the agar medium by metabolic products which had diffused outwards from the primary growth. However, it was shown that this was not the case and that the zone was indeed populated by organisms which, possessing power of movement, had been able to pass outwards from their places of origin. This was proved by the fact that material removed from points near the periphery of the zone and inoculated into fresh medium readily gave rise to active subcultures. Next it was noted that, apart from the question of whether or not the giant colony of an *Acetobacter* species on a plate of semi-solid wort-agar shows the presence of motile cells, it exhibits characteristics which either are specific for that particular species or are typical for a group of several species of which that particular one is a member. Janke, as mentioned above, had already demonstrated the usefulness of the giant colony in this connection, but it was seen that the use of a semi-solid medium permits the development of growth forms which cannot come into being under Janke's conditions of cultivation. As an example of the observations made during the course of the present work it was seen that, out of 18 species which were studied, *A. capsulatum* gave giant colonies entirely unlike any of those given by the other species, whereas, on the other hand, *A. suboxydans*, *A. mobile* and *A. turbidans* gave giant colonies which showed certain features in common, and which could be placed in one group along with the giant colonies of certain other species which behaved in a similar manner.

Janke photographed his colonies by transmitted light against a strongly illuminated background and he states that he used a magnification of 11 $\times$, obtained with a N-00 objective and a N-2 projection ocular. The writers have found that when applying

a dark field technique the outer zone of growth formed by the cells of motile species which have moved away from the site of inoculation can be clearly discerned. The advantage of this can be demonstrated by comparing the photograph of a motile species taken with top lighting, in which the outer zone shows up but dimly, with the photograph of the same colony taken with dark field illumination for, in the latter case, the outer zone shows up to an outstanding degree (see, e.g., the photographs of *Acetobacter viscosum*, given later).

EXPERIMENTAL

Method of Cultivating Giant Colonies of Acetobacter Species.—Preliminary experiments to ascertain the best conditions were carried out with *A. suboxydans*, a motile species.

(1) Wort-agar prepared with only 0.5 per cent. of agar was first tried. This is the lowest concentration of agar which, at room temperature, gives to a medium a consistency which enables it to set in a petri-dish and, subsequently, to be handled without destruction of the typical structure of the colony grown on it. The main colony of *A. suboxydans* grown on 0.5 per cent. wort-agar had the following appearance:—The centre was circular, showing rhizoid projections with very many details, the projections being greatly extended. The zone was very wide-spreading and strongly developed.

(2) Using 0.8 per cent. of agar in wort the main colony was circular at the centre and of the same size as that in (1), but it showed projections which were far less extended and which displayed much less detail than those in (1). The zone was not developed at all.

(3) Using 1 per cent. of agar in wort the main colony had a circular centre and was of the same size as that in (1), but there were no rhizoid projections nor was any zone detected.

Finally, it was decided to use wort containing 0.7 per cent. of agar, since this gave a medium which was just a little firmer than that mentioned in (1), and was, therefore, easier to manipulate, whilst at the same time it permitted growth of secondary projections from the primary circular colony and allowed also development of a well-marked zone of growth. Some time later,

however, when using agar from a different source, it was found that less than 0.7 per cent. was sufficient to give good results; evidently, therefore, a hard and fast rule cannot be laid down respecting the exact concentration of agar, but it will depend on the quality of the material available.

The wort was of sp.gr. 1.036 and the medium was clarified with egg-albumin. Inoculation was performed by means of a platinum wire such as is used for making stab-cultures, but with the difference that the wire was bent at an angle near to its free point. This was dipped into an active young culture in liquid malt-wort and it was then allowed just to touch the surface of the 0.7 per cent. wort-agar which, previously, had been poured into a petri-dish and allowed to set there. The advantage of the angle-bend near the end of the wire lies in the fact that it enables the inoculation to be carried out by holding the handle (in which the platinum wire is fixed) at a normal angle, whereas, if there is no angle near the end of the platinum wire, the latter has to be brought down vertically on to the surface of the agar, in which case it is very difficult to just touch the agar without allowing the end of the wire to penetrate below the surface. Should the wire penetrate the agar the resultant colony will not be of typical character. Janke used a fine mapping pen to perform the inoculations, but in our hands the use of a platinum wire as described above gave better results. All the species, with the exception of *A. orleanense*, *A. rancens* and *A. ketogenum* (*sp. nov.*), were allowed to grow on the agar for 14 days, the temperature of the room being 16° to 18° C. In the cases of these three species the temperature of the room during incubation was 17° to 21° C. and in a subsequent test with the same three species it was 18° to 20° C., the weather being very warm at the time. The temperature range 16° to 18° C. gives the best results.

In order to obtain photographs of the giant colonies (*a*) with dark field illumination and (*b*) with top lighting, a special apparatus was devised for use with the camera. This will form the subject of a separate communication which it is hoped shortly to publish elsewhere.

Terms used to describe the Giant Colonies.—The different species which yield giant colonies of the same type have been placed in groups: thus the group of pellicle formers and the group of zone formers. Some

species give colonies which do not fall into one of the groups, but exhibit such distinct differences from all others that they must be regarded as individual types.

- | | |
|--------------------------|---|
| I. General appearance | { (a) Delicate or abundant growth.
(b) With or without zone. |
| II. Type of Colony | { Belonging to one of the following groups:—
<i>A. acetigenum</i> group (pellicle formers).
<i>A. acetosum</i> group.
<i>A. suboxydans</i> group (zone formers).
Or a distinctive type of its own, as exemplified by the following, all of which are different:—
<i>A. aceti</i>
<i>A. acidum-polymyxa</i>
<i>A. capsulatum</i>
<i>A. gluconicum</i> . |
| III. Main central growth | { (c) Regular or otherwise.
(d) Shape.
(e) Size (diameter, mm.).
(f) Structure (observed microscopically).
(g) Elevation (flat, raised, dome-shaped or effuse).
(h) Opaque or translucent.
(i) Consistency.
(j) Colour shading.
(k) Shiny or dull.
(l) Iridescence of marginal part.
(m) Margin of central growth (observed microscopically and described as entire, undulate, lobate, erose, filamentous or curled).
(n) Structure of marginal area (observed microscopically). |
| IV. Zonal growth | { (o) Delicate.
(p) Flat.
(q) Shiny or dull.
(r) Translucent.
(s) Margin of zone (observed microscopically).
(t) Structure of marginal zone (observed microscopically).
(u) Size of zone (diameter, mm.). |

Descriptions of Giant Colonies.—*A. acetigenum* (Henneberg) Bergey *et al.* (N.C.T.C. 5346).

- I. Colony of coherent consistency. (a) Abundant growth, (b) without zone.
- II. Representative of the group of pellicle-formers.
- III. (c) Regular, (d) circular, (e) 16 mm., (f) no structure, (g) high dome-shaped, (h) opaque, (i) tough and coherent and can be caused to slide intact over the surface of the agar, (j) centre yellowish, outer part milky, (k) shiny, (l) not iridescent, (m) filamentous and coherent, (n) marginal area evenly covered.

A. kützingerianum (Hansen) Bergey *et al.* (N.C.T.C. 3924).

- I. Colony of coherent consistency. (a) Abundant growth, (b) without zone.
- II. Belonging to the *A. acetigenum* group (pellicle-formers).
- III. (c), (d), (e), (f), (h), (i), (j), (k), (l), (m), and (n) as for *A. acetigenum*, (g) dome-shaped.

A. pasteurianum (Hansen) Beijerinck (N.C.T.C. 613).

- I. Colony of coherent consistency. (a) Small colony and somewhat delicate growth, (b) with zone.
- II. Belongs to *A. acetigenum* group (pellicle-formers) but, unlike the latter species, shows a zone.
- III. (c) Fairly regular, (d) ellipsoidal, (e) 1 × 3 mm. (f) showing nodules, (g) flat, (h) opaque, (i) coherent and can be caused to move intact over the agar, (k) shiny, (l) not iridescent, (m) not distinct, (n) not distinct.
- IV. (o) Delicate, (p) flat, (q) dull, (r) semi-transparent, (s) density gradually fading away, (u) 5 mm.

Additional remarks:—Motility was not observed microscopically.

Bergey *et al.* record: "Motility variable."

A. xylinum (Brown) Bergey *et al.* (N.C.T.C. 4112).

- I. Colony of coherent consistency. (a) Abundant, (b) without zone.
- II. *A. acetigenum* group (pellicle-formers).
- III. (c) Regular, (d) circular, (e) 7 mm., (f) no structure, (g) dome-shaped, (h) opaque, (i) tough and coherent, can be caused to slide intact over the surface of the agar, (j) centre yellowish, outer part milky, (k) shiny, (l) not iridescent, (m) filamentous and coherent, (n) marginal area evenly covered.

A. acetosum (Henneberg) Bergey *et al.* (N.C.T.C. 2224).

- I. Opaque structureless colony of butter-like consistency and without zone.
- II. *A. acetosum*.
- III. (c) Nearly regular, (d) circular, (e) 4 mm., (f) no structure, (g) slightly raised, (h) opaque, (i) butyrous (butter-like), (j) shiny, (l) iridescent, (m) undulate, entire, but at one place on the margin a number of daughter colonies developed. This has been observed also in one or two other cases.

A. acetosum (Strain B).

- I. Exactly like *A. acetosum* (N.C.T.C. 2224).
- II. *A. acetosum*.
- III. (c), (d), (f), (g), (h), (i), (k), (l) as for *A. acetosum* (Henneberg) Bergey *et al.* (N.C.T.C. 2224); (e) 3 mm., (m) entire, (n) marginal area evenly covered.

A. acetosum (Strain Y).

- I. Exactly like *A. acetosum* (N.C.T.C. 2224) and *A. acetosum* (Strain B).
- II. *A. acetosum*.
- III. (c) Fairly regular, (d) ellipsoidal, (e) 2 to 4 mm., (f) no structure, (g) flat, (h) opaque, (i) butyrous, (k) shiny, (l) iridescent, (m) entire, (n) marginal area evenly covered.

A. ascendens (N.C.T.C. 4937).

- I. Opaque, structureless colony, of butter-like consistency and without zone.
- II. *A. acetosum* group.
- III. (c) Regular, (d) circular, (e) 5 mm., (f) no structure, (g) slightly raised, (h) opaque, (i) butyrous, (k) shiny, (l) iridescent, (m) entire, (n) marginal area evenly covered.

A. rancens (N.R.R.L. B65).

- I. Butyrous, structureless colony. (a) Medium growth, (b) devoid of zone.
- II. *A. acetosum* group.
- III. (c) Very lobate, (d) circular, (e) 20 mm., (f) inner part no structure, outer part concentric lines, (g) nearly flat, (h) opaque, (i) butyrous, (j) even whitish tint, (k) slightly shiny, (l) not iridescent, (m) entire, (n) evenly covered.
A strain of *A. oxydans* isolated recently by one of us (D.K.) from an East African vinegar brew and designated *A. oxydans* var. *africanum* was also found to yield a giant colony of the type produced by *A. acetosum* and a description of this, and also of the giant colony of *A. acetosum* var. *nairobiense*, is reserved for a forthcoming communication dealing with acetic acid bacteria from an East African vinegar brewery.

A. suboxydans (N.C.T.C. 7069; A.T.C.C. 621).

- I. Colony of butter-like consistency. (a) Delicate, (b) with zone.
- II. *A. suboxydans*.
- III. (c) Regular, (d) circular. The colony is fairly distinct in contrast with the zone, (e) 2 mm., (f) irregular, not rhizoid, (g) flat, (h) opaque, (i) butyrous, (k) shiny, (l) not iridescent, (m) not distinct, (n) not distinct.
- IV. (o) Delicate, (p) flat, (q) dull, (r) semi-transparent, (s) density gradually fading to completion, (t) marginal area not evenly covered, (u) 19 mm.

A. suboxydans (N.C.T.C., 6723, N.R.R.L. B72).

This colony was exactly as N.C.T.C. 7069 except that (e) was 3 mm. and (u) was 15 mm.

Giant Colonies of *Acetobacter* Species (on semi-solid wort-agar).

Magnification $\times 2.9$. Projection lens Leitz 64 m.m. f. 4.5



FIG. I
A. aceti. Top lighting



FIG. II
A. aceti. The same colony photographed
with dark field illumination



FIG. III
A. ascendens. Dark field illumination



FIG. IV.
A. capsulatum. Dark field illumination



FIG. V
A. turbidans. Dark field illumination

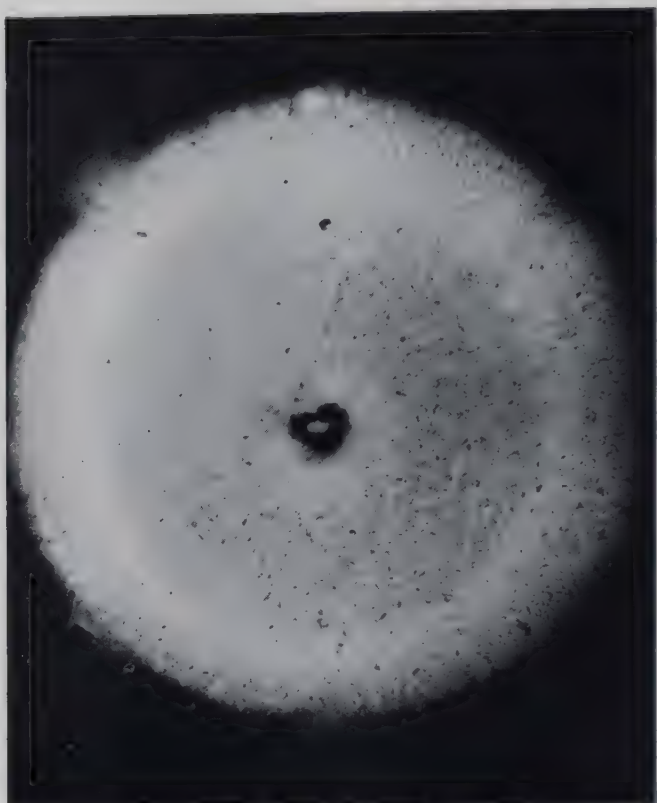


FIG. VI
A. mobile. Dark field illumination



FIG. VII
A. viscosum. Top lighting



FIG. VIII
A. viscosum. The same colony photographed with dark field illumination

A. suboxydans H.3.

This strain was isolated from a sour beer by one of us (T.K.W.) and gave a giant colony exactly like those of the two other strains of this species with the exception that it possessed a very large zone, (u) being 25 mm.

A. ketogenum Walker and Thomas, a new species isolated recently from top-fermentation beer was found to yield a giant colony of very distinctive appearance and possessing a very large zone. It belongs to the *A. suboxydans* group of zone-formers and will shortly be described in a separate communication.

A. mobile Tosic and Walker (N.C.T.C. 6428).

- I. Colony of butter-like consistency (a) Very delicate, (b) with zone.
- II. *A. suboxydans* group.
- III. (c) and (d) Regular and circular and distinct in contrast with the zone, (e) 2 mm., (f) irregular, (g) flat, (h) opaque, (i) butyrous, (k) dull, (l) not iridescent, (m) and (n) not distinct.
- IV. (o) Very delicate, (p) flat, (q) dull, (r) semi-translucent, (s) intensity gradually fading away, (t) uneven and extremely thin, (u) 26 mm.

A. orleanense (N.R.R.L. B55).

- I. Colony of butter-like consistency (a) Delicate, (b) presence of zone uncertain.
- II. Probably *A. suboxydans* group.
- III. (c) Regular, (d) circular, (e) 24 mm., (f) indistinct, (g) flat, (h) opaque, (i) butyrous, (j) off-white, (k) slightly shiny, (l) not iridescent, (m) and (n) not distinct.
- IV. The zone was difficult to observe with certainty but appeared to be flat, dull and semi-translucent, with a margin, the density of which gradually faded away. Its diameter appeared to be about 32 mm.

A. turbidans Cosbie, Tosic and Walker (N.C.T.C. 6249)

- I. Butter-like consistency. (a) Delicate; (b) with zone.
- II. *A. suboxydans* group.
- III. (c) Regular, (d) circular. The regular circular growth of the centre of the colony is most distinct in contrast with the zone, (e) 5 mm., (f) no structure, (g) slightly raised, (h) opaque, (i) butyrous, (k) shiny, (l) not iridescent, (m) indistinct, (n) indistinct.
- IV. (o) Very delicate, (p) flat, (q) dull, (r) opaque, (s) density gradually fading away, (t) marginal area not evenly covered, (u) 21 mm.

A. viscosum Baker, Day and Hulton (N.C.T.C. 6426).

- I. Colony of butter-like consistency. (a) Very delicate, (b) with zone.
- II. *A. suboxydans* group.
- III. (c) and (d) Regular rhizoid shape very distinct in contrast with the zone, (e) 2 mm., (f) rhizoid, (g) flat, (h) opaque, (i) butyrous, (j) shiny, (l) not iridescent, (m) and (n) not distinct.

- IV. (o) Delicate, (p) flat, (q) dull, (r) semi-translucent, (s) density gradually fading away, (t) marginal area not evenly covered, (u) 13 mm.

A. aceti (N.C.T.C. 6423).

A strain isolated by Walker and Tosic and given in the Lister Institute List as *A. aceti* I.

- I. Colony of butter-like consistency. It was partly opaque, partly transparent, showing a regular structure and no zone.
- II. Type of its own.
- III. (c) Regular, (d) circular, (e) 8 mm., (f) the colony has a most distinct centre and a regular lobate pattern, (g) convex. The outer circle of the main colony is opaque and the inner main part is yellowish, transparent and shiny, (l) not iridescent, (m) entire, (n) marginal area evenly covered.

A. acidum-polymyxa Walker and Tosic (N.C.T.C. 6429).

The colony is of butter-like consistency and is opaque, showing under the microscope a typical structure without zone. It is a type of colony of its own and quite unlike any of those given by any other species which we have examined. The main central growth of the colony is perfectly regular and circular, 5 mm. in diameter, convex, opaque, shiny, not iridescent. The margin of the central growth is entire. Radial lines regular in character, are visible in the outer parts.

A. capsulatum Shimwell (N.C.T.C. 4943).

- I. Colony of slimy consistency, perfectly transparent and nearly structureless. (a) Abundant, (b) no zone.
- II. Type of its own.
- III. (c) and (d) Fairly regular and ellipsoidal, (e) 28 × 18 mm., (f) some irregular structure, (g) convex, (h) perfectly transparent, nearly "water-clear," (i) slimy, (k) shiny, (l) not iridescent, (m) entire, (n) marginal area evenly covered.

A. gluconicum Hermann (N.C.T.C. 4739).

- I. Colony of butter-like consistency. (a) Delicate, (b) distinct though very faint zone.
- II. A type of its own and readily recognized.
- III. (c) and (d) nearly regular and circular as seen against the very feeble zone, (e) 5 × 4 mm., (f) not regular, (g) nearly flat, (h) opaque, (i) butyrous, (j) pink, this organism is the only pigment-former encountered amongst the organisms described in this communication; (k) shiny, (l) not iridescent, (m) and (n) both indistinct.
- IV. The zone was scarcely visible and it was doubtful whether or not it was present round some colonies.

Particulars Respecting the Plates.—Figures I and II show the same colony of a strain of *A. aceti* (N.C.T.C. 6423) photographed with the two methods of lighting. The characteristic structure of the colony is clearly depicted

in these photographs. This strain is a harmful contaminant of beer.

Figure III represents the Non-Zone-Forming Group. This species (*A. ascendens*) is harmless in beer.

Figure IV shows *A. capsulatum*, which is a beer disease organism giving a very typical transparent giant colony. In this plate it will be noted that one side of the colony has been disturbed. This disturbance was caused by the removal of an infection which grew adjacent to the colony and was cut off prior to taking the photograph.

Figures V and VI show how dark field illumination reveals the large zones of growth round the colonies of *A. turbidans* and *A. mobile*, respectively. These two species are very motile. Both are very harmful beer infections.

Figures VII and VIII are included in order to reveal how much better the zone of a motile species shows up when photographed with dark field illumination than with top lighting. Both plates depict the same colony seen under the two conditions. *A. viscosum* is harmful in beer.

The colonies are shown at a magnification $\times 2.9$. Projection lens Leitz 64 mm., f. 4.5.

SUMMARY

- (1) Janke cultivated *Acetobacter* species as giant colonies on a solid medium of normal consistency and noted that some species gave forms of growth which were highly characteristic and thus assisted diagnosis of types. The present authors have found that by using a semi-solid medium the usefulness of the method is greatly increased, for in the majority of cases (exceptions being most of the pellicle-forming species) motility when it occurs can be discovered as a zone around the main colony. *A. pasteurianum*, a pellicle-former, forms giant colonies which show zones.
2. The semi-solid medium best suited for the purposes of the present experiments

was found to be wort-agar containing 0.7 per cent. of agar-agar. The exact percentage of agar to give optimum results depends, however, on the quality of the agar used and in some cases may be a little less than 0.7 per cent.

3. The authors have also found that the use of this semi-solid medium permits the development of characteristic growth forms which do not come into being under Janke's conditions of cultivation.
4. It has also been observed that in some cases it is a great advantage, from the point of view of ease of identification of types, to photograph the colonies with dark field illumination. In other instances photographs taken with top lighting reveal very characteristic formations.
5. Employing this technique for the examination of 18 species of *Acetobacter*, it was seen that they could be grouped as follows:—

A. acetigenum, *A. kützingianum*, *A. pasteurianum*, and *A. xylinum*, all of which form coherent pellicles on normal liquid media, gave giant colonies of the same type.

The giant colonies of *A. acetosum*, *A. ascendens*, *A. rancens* and a strain of *A. oxydans* from an East African vinegar brewery, were all similar and constituted a second group.

A third group comprised the giant colonies of *A. suboxydans*, *A. ketogenum* (*sp. nov.*), *A. mobile*, *A. orleanense*, *A. turbidans* and *A. viscosum*, each of which possessed a zone formed by motile cells.

Species which formed giant colonies each of which was a type of its own and unlike any of the others, were *A. aceti*, *A. acidum-polymyxa*, *A. capsulatum*, and *A. gluconicum*.

College of Technology,

University of Manchester.

1st December, 1947.

NITROGEN COMPOUNDS OF WORT AND BEER. PART II

THE FRACTIONATION OF THE WORT AND BEER NITROGEN COMPOUNDS BY PRECIPITATION WITH PHOSPHOTUNGSTIC ACID

By L. R. BISHOP, M.A., D.Sc.

(Received by the Chairman: 9/12/47)

In studying the nitrogen compounds of wort and beer, the first requirement is for an understanding of the broad general types of nitrogen compounds in wort and of their approximate proportions. An attempt to provide such a separation has been made by adding phosphotungstic acid to wort in a series of regularly increasing quantities, and separating and analyzing the fractional precipitates.

The results indicate precipitation in a series of jumps, with corresponding jumps in the composition of the precipitates. Fully-built-up proteins have a very small proportion of free amino nitrogen, whereas simple mono-amino-acids yield 100 per cent.; thus the complexity of the precipitates can be assessed from these values. Indications have been obtained of compounds containing approximately 1, 2, 4, 8, 15-16 and around 30 per cent. of free amino nitrogen.

In beer the corresponding complex groups have been found in similar amounts with the simpler compounds reduced or absent owing to absorption by yeast.

The results obtained have been supported by preparation and analysis of the complex wort nitrogen compounds.

THE general background to the present studies has already been summarized in an Introduction to this Series (*this Journ.*, 1943, 168). From this it will be seen that the two immediate problems appear to be those of (a) sorting the nitrogen compounds into groups of different molecular size—from proteins down to single amino-acids and (b) determining the chemical nature more particularly of the simpler nitrogen compounds. The second problem is left for later treatment and the present paper is concerned with the problem of sorting out nitrogen compounds in respect of molecular size.

Members of the most complex group, the fully-built-up proteins, play an important part in wort and in beer. Schjerning pointed out many years ago that the albumins would be coagulated on boiling and that only globulins would be expected to survive. This suggestion has been established by the work of E. Sandegren (*Proc. Europ. Brew.*

Conv., 1947, 28), who has shown that only the characteristic β globulin of barley survives in wort and beer. Though present only in minute amounts, it plays an important part, for he has shown that this protein is the one responsible for haze. A method for the estimation of the small amounts of fully-built proteins in wort and beer has been suggested (*this Journ.*, 1944, 224).

This true protein fraction of the nitrogen compounds of wort is therefore receiving attention. It is important, but constitutes only a small proportion of the nitrogenous compounds, and what is wanted is a study of the main bulk of these compounds which exist chiefly in less complex forms in wort and beer. As a start in this direction the present study gives the results of a broad, semi-quantitative fractionation.

A number of methods for the fractional estimation of the wort nitrogen compounds have been published in the past, for instance

that of Schjerning, in which precipitants such as stannous chloride, mercuric chloride, ferric acetate and uranium acetate are used. Unfortunately, as far as the author is aware, no criterion has been applied to test the homogeneity or molecular size of the fractions precipitated by these methods, so that their nature is indeterminate. The consequent misleading nature of the Schjerning results was shown many years ago by H. T. Brown in *Trans. Guinness Research Lab.* (1903-6).

In the course of studies of different precipitants it was found by the author in 1935 that the acidic precipitants offer a useful means of fractionating the nitrogen compounds of wort according to molecular size. Of these precipitants, phosphotungstic acid removed the greatest amount of nitrogen from wort and was consequently used in a method in which a long series of regularly increasing doses is added, as explained later in the text.

With the series of fractions separated by this means it would be possible to use ultracentrifuging or electrophoresis to measure their molecular complexity, but the specialized apparatus required was not available. Consequently recourse was had to the "Amino index" of the separate fractions as the best available measure of their complexity.

Horace Brown in his important preparative work on the nitrogen compounds (*loc.cit.*) had initiated the use of the "Amino-index" (the ratio of free amino to total nitrogen) as a guide to the complexity of his wort nitrogen fractions, but very little use appears to have been made of it. At one end of the scale of complexity stand the single mono-amino acids with 100 *per cent.* free amino nitrogen and at the other end there are the fully-built-up proteins giving from 1 to 5 *per cent.* amino nitrogen. This chemical measure has been applied to estimate the average order of physical size of any fraction of nitrogen compounds separated from wort in the present and subsequent studies. It is analogous to the measurement of the reducing power of carbohydrates as a measure of their complexity.

With the help of these methods, evidence has been obtained of distinct fractions of nitrogen compounds in wort, which are similar between different worts according to the "principle of regularity," and direct evidence has been obtained that it is only the simpler nitrogen compounds which can be absorbed by yeast.

PRECIPITATION OF WORT NITROGEN COMPOUNDS BY PHOSPHOTUNGSTIC ACID

Phosphotungstic acid was originally used towards the end of the last century as a precipitant for proteoses and peptones, but more recently this use has been obscured by its use as a precipitant for the basic amino-acids in the Hausmann method. In his work Horace Brown used a high concentration of phosphotungstic acid (in conjunction with sulphuric acid) to precipitate as much as possible of the nitrogen compounds of wort which were then fractionated by other means. In one attempt to subfractionate he added half the usual quantity and found little evidence of fractionation.

The origin of the present work lay in the observation that the phosphotungstates of the more complex nitrogen compounds are much more insoluble than those of the less complex, so it was found that, if *e.g.* one-fifth or one-tenth of the total phosphotungstic acid required is used, then good fractionation is obtained. That is, the compounds formed by the complex nitrogen compounds with phosphotungstic acid are very insoluble, whereas to precipitate the simple ones high concentrations of both nitrogen compounds and phosphotungstic acid are necessary, and for the same reason the precipitates are readily redissolved on washing. This is illustrated in Table I, which gives the fractional precipitation of an alcohol-water extract of malt, by phosphotungstic acid. The extract had been concentrated and the alcohol removed by distillation, *in vacuo*. One hundred ml. were taken, sulphuric acid was added to a concentration of 5 *per cent.* and 1.0 gm. of phosphotungstic acid was added in solution.* The precipitate was removed and a further 8.0 grms. of phosphotungstic acid were added and the second precipitate removed. The final solution and the two precipitates were then analyzed.

Similar results were obtained by taking wort without adding sulphuric acid and adding for the first precipitate, 5.0 grms. phosphotungstic acid and, for the second precipitate, sulphuric acid to 10 *per cent.*

Thus the indications were that phosphotungstic acid could be used as a method for

* All phosphotungstic acid used was the 1 : 24 ($P_2O_5 : WO_3$) acid and it was always added in the form of an aqueous solution containing 1 gm. (P_2O_5 , $24WO_3$, $24H_2O$) *per ml.* The acid is conveniently referred to as "p.t.a."

TABLE I.

FRACTIONATION OF WORT NITROGEN COMPOUNDS BY PHOSPHOTUNGSTIC ACID.

	Percentage of Wort Nitrogen.	Amino- nitrogen Index.
First precipitate (1.0 grm. p.t.a.) ..	<i>Per cent.</i> 23.6 (119 mgrms.)	<i>Per cent.</i> 7.7
Second precipitate (8.0 grms. p.t.a.) ..	17.4 (88 mgrms.)	17.8
Final Solution ..	59.0 (298 mgrms.)	30.0

precipitating first the most complex nitrogen compounds of wort and then successively those of lesser and lesser complexity, and so could give a broad general outline of wort nitrogen composition.

As an analogy we may take the stepwise addition of hydrochloric acid to a solution of the acetates of silver, lead and sodium. The first small additions would cause precipitation of silver then the lead would follow, but to precipitate the sodium chloride it would be necessary to pass in gaseous hydrochloric acid to raise the concentration sufficiently.

FIRST EXPERIMENT

The experiment was made on a concentrated wort of 1150° sp. gr. and the corresponding beer after similar concentration. Each of these was precipitated with a series of regularly increasing quantities of phosphotungstic acid, each of the precipitates being then refractionated as a test of its purity and individuality.

The results are not as exact as those obtained later, but they indicate significant amounts of two groups of complex nitrogen compounds in both beer and wort. These are fairly pure in the beer precipitates whilst the wort precipitates are more contaminated owing to the difficulty of freeing the sticky precipitates from wort and the need for quantitative recovery which precludes effective washing. Knowing this, the free amino indices of the first two main experiments are to be taken only as rough guides, because traces of low molecular compounds will raise the apparent free amino index of the complex compounds.

The details of the first experiment were as follows. One litre of hopped wort of 1075° sp. gr. was concentrated to 500 ml. and analyzed. An equal volume was fermented by yeast,

filtered, and similarly concentrated in vacuo to 500 ml. An incidental advantage of this was that alcohol was removed from the fermented portion. To each solution p.t.a. was added gradually, with stirring, in the quantities given in Table II.

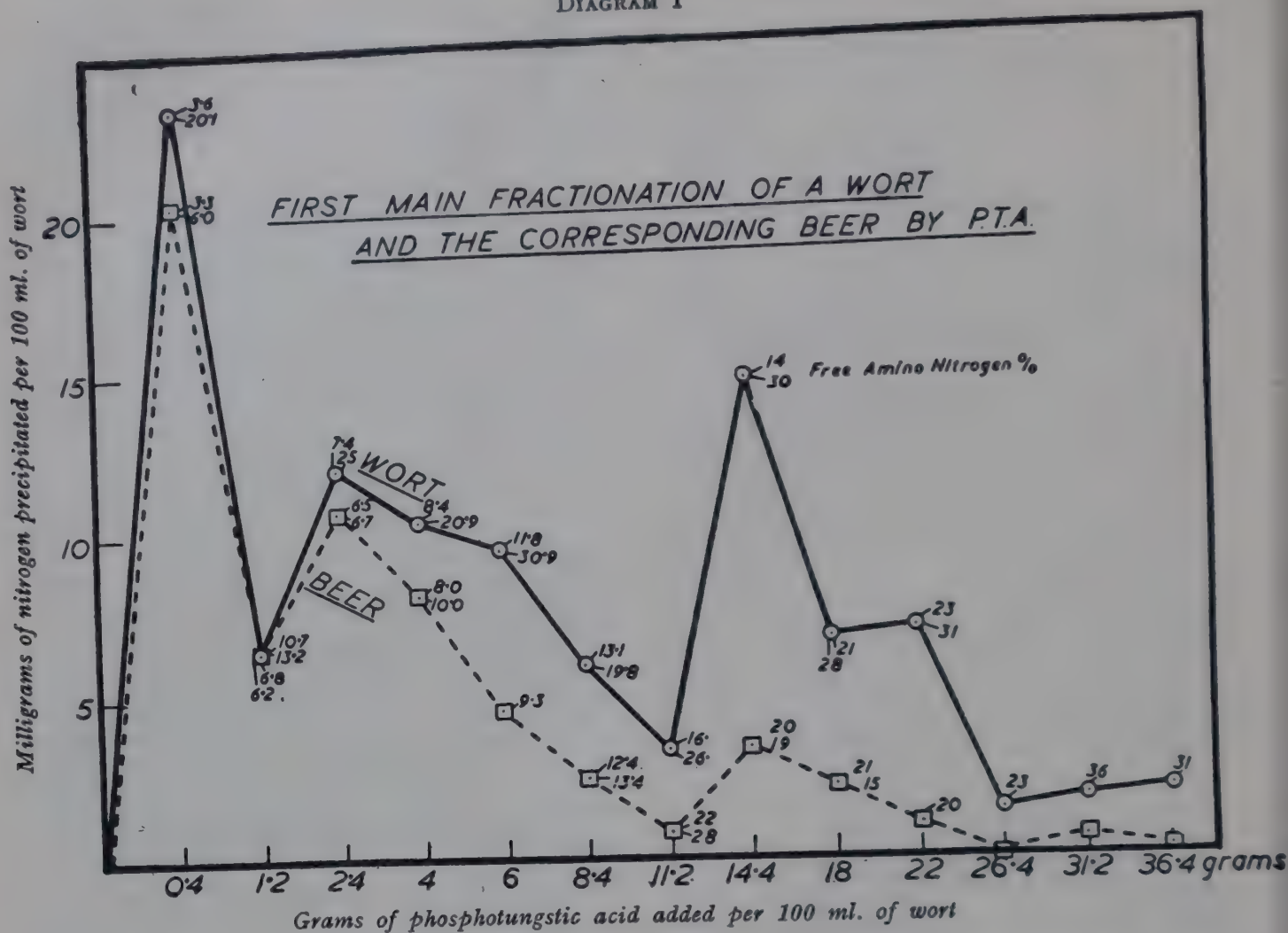
The solution was allowed to stand overnight after each addition and the precipitate separated by centrifuging. With these partial precipitations the separation was difficult and required prolonged centrifuging. Each precipitate was collected and filtered as dry as possible on a Jena sintered glass funnel and then dissolved in water with the addition of the minimum amount of sodium bicarbonate and the solution made to a definite volume (usually 50 ml.). Total nitrogen content and free amino nitrogen by the Van Slyke method of aliquot portions were determined. An aliquot portion of the remainder was cautiously precipitated by the gradual addition of hydrochloric acid, until it was judged that approximately half the nitrogen had been precipitated. The precipitate was then removed and dissolved, constituting sub-fraction (a), while the residual solution constituted sub-fraction (b). The total nitrogen content, free amino nitrogen and amide nitrogen content were determined in each of these.

The results are set out in Table II and Diagram I. They show that, in the wort, with the increase of p.t.a. concentration, the proportion of free amino to total nitrogen increased—in other words, more and more simple compounds were successively precipitated. Further, the precipitation tended to occur chiefly when certain concentrations of precipitant were reached, indicating the existence of discrete groups of nitrogen compounds in wort. In the beer these groups corresponded in the complex parts while the simpler ones were missing—indicating, as is now generally accepted, that the yeast takes the simpler compounds only during its growth. In beer the amounts of the complex fractions are rather lower than the corresponding fractions from wort. The contamination of the latter with less complex nitrogen compounds (shown by the re-fractionations) will account for this.

SECOND EXPERIMENT

The second experiment followed similar lines except that as much nitrogen as possible

DIAGRAM I



was first removed by salting out with magnesium sulphate. This had two objects: one was to see whether this entirely different method showed similar constituents (which it did, as shown below), and the other was to increase the proportion of nitrogen precipitated when the p.t.a. was added afterwards in increasing steps. The amount precipitated was increased further by successive addition of concentrated hydrochloric acid. About 65 per cent. of the wort nitrogen and 80 per cent. of the nitrogen of its beer were precipitated. Broadly, the same free amino nitrogen percentages were found as in the first experiment.

The details of this second experiment were as follows:—500 ml. of concentrated wort was used of 1220° sp. gr. and a corresponding amount of wort diluted, fermented under brewery conditions and reconcentrated.

As stated, the wort was first saturated with magnesium sulphate and the precipitate removed. Subsequent precipitates were

obtained by additions of p.t.a. in increasing amounts, followed by additions of concentrated hydrochloric acid. Rather larger fractions were taken at each precipitation and they were not subfractionated. The results are given in Table III.

THIRD EXPERIMENT

In the first two experiments attempts had been made to do two things at once, that is, to separate the different groups of nitrogen compounds quantitatively and to prepare them pure for analysis. In further work it seemed desirable to separate these two aspects. Consequently, the next step was to make two different experiments and measure only amounts of total nitrogen precipitated in one set of experiments, while in another the washed precipitates were analyzed and their composition studied. At the same time improvements were introduced in using a centrifuge operating at a higher speed which gave more effective separation of the p.t.a.

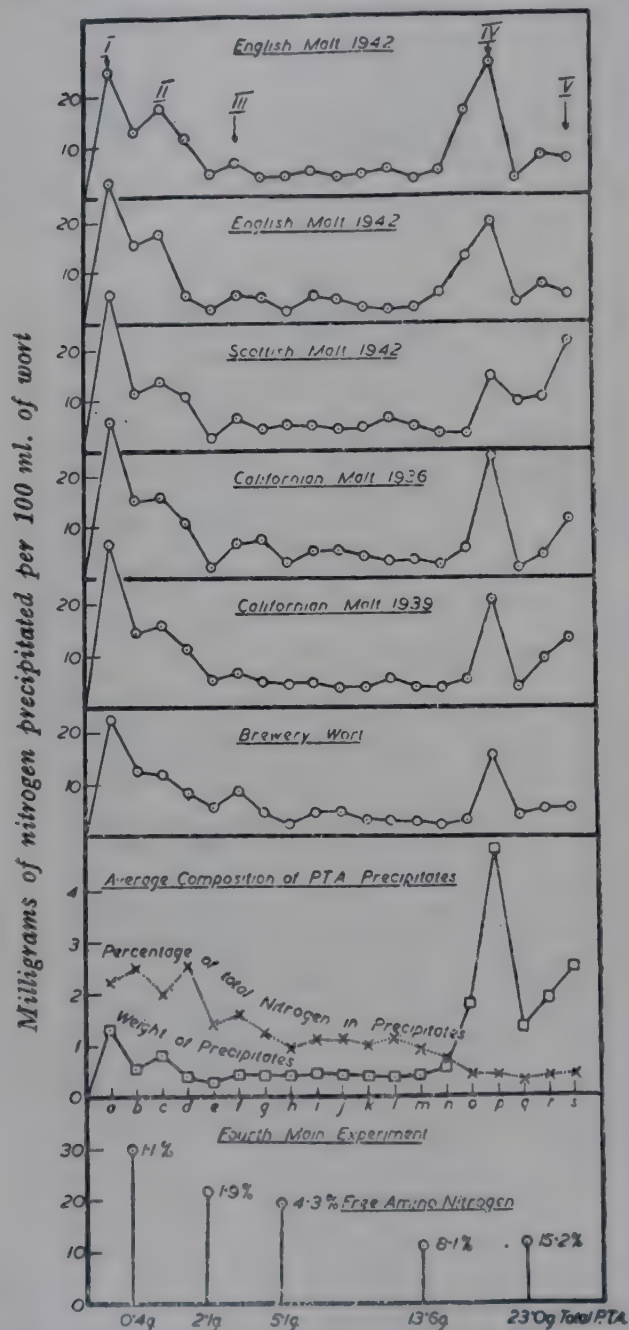
TABLE II.
FIRST WORT AND BEER FRACTIONATION EXPERIMENT
Quantities of total nitrogen are all in mgrms. *per* 100 ml. of the original wort.

P.T.A. Total Addi- tion.	WORT.						BEER.					
	Main Fraction.			Subfractions.			Main Fraction.			Subfractions.		
	Total Nitro- gen.	T.N. as <i>per cent.</i> original wort N.	Amino <i>per cent.</i> of T.N.	T.N.	Amino <i>per cent.</i> of T.N.	Amide <i>per cent.</i> of T.N.	Total Nitro- gen.	T.N. as <i>per cent.</i> original wort N.	Amino <i>per cent.</i> of T.N.	T.N.	Amino <i>per cent.</i> of T.N.	Amide <i>per cent.</i> of T.N.
Grms. + 2	23.33	13.2	8.2	15.60 7.43	3.6 20.1	9.0 —	20.30	11.5	3.4	11.61 7.18	3.3 6.0	11.5 —
+ 6	6.36	3.6	13.2	1.96 4.18	10.7 13.2	9.2 —	6.27	3.6	5.9	1.77 4.40	6.8 6.2	10.5 —
+ 12	11.98	6.8	11.8	9.84 2.62	7.4 25.7	8.0 —	10.70	6.1	7.0	6.87 4.07	6.5 6.7	7 —
+ 20	9.24	5.2	14.2	5.16 4.36	8.4 20.9	11.0 —	8.12	4.6	7.6	5.60 2.62	8.0 10.0	6 —
+ 30	9.50	5.4	18.6	5.08 4.29	11.8 30.9	10.0 —	4.52	2.6	8.6	3.17 1.52	9.3	6
+ 42	5.90	3.4	19.0	1.66 4.56	13.1 19.8	11 —	2.42	1.4	9	1.09 1.23	12 13	— —
+ 56	3.26	2.0	15.9	1.77 1.56	16 26	11 —	0.67	0.4	11	0.34 0.46	22 28	12 —
+ 72	14.66	8.3	28.5	3.00 11.52	13.5 29.6	12 —	3.29	2.0	19	0.83 2.67	20 19	10 —
+ 90	6.61	3.8	25.7	3.85 2.42	21 28	— —	2.12	1.2	21	1.29 0.77	21 15	6 —
+ 110	6.83	3.9	31.0	1.55 5.25	23 31	9.0	0.92	0.5	20			
+ 132	1.30	0.7	23.0	—	—	—	0.04	—	—	—	—	—
+ 156	1.72	1.0	36.0	—	—	—	0.47	0.3	17	—	—	—
+ 182	1.92	1.1	31.0	—	—	—	79.0*	44.9*	—	—	—	—
Residue	57.18	32.5	36.0	—	—	0.3	30.85	17.5	9.4	—	—	1
Original Wort	176.1	—	24.6	—	—	—	—	—	—	—	—	—

* Total Nitrogen in yeast.

precipitates. These were also treated differently to ensure more complete removal of wort and the more accurate Van Slyke Blood Gas Analysis Apparatus was used for

DIAGRAM II
THIRD AND FOURTH EXPERIMENTS ON FRACTIONATION OF WORTS WITH PHOSPHOTUNGSTIC ACID



Phosphotungstic acid—addition per 100 ml. of wort

In Diagrams I and II the amounts of wort nitrogen precipitated have been plotted against the linear increase in amount of phosphotungstic acid added at each step, but against these points the total addition figure has been put, as it is more informative and allows comparison to be made between the two diagrams. Percentages of free amino on total nitrogen for the different fractions are inserted in the Fourth Main Experiment diagram.

measuring the free amino nitrogen instead of the older Van Slyke amino nitrogen apparatus.

Six worts were chosen to cover a wide range of composition. Five were made by laboratory mashes at the rate of 300 grms. of malt to 700 ml. of water, the sixth was a brewery wort (the wort 19 of the Wort Analysis Series). Two of the laboratory mashes were of Californian malt and the other three were British malts. One of the latter was a good malt, one was high in nitrogen and one was badly modified.

The boiled, unhopped worts were concentrated *in vacuo*, made to 1150° sp. gr., centrifuged at high speed and filtered bright before analysis. Total nitrogen content was determined in the worts and duplicate 100 ml. portions of each taken for the main experiment in 100 ml. centrifuge tubes.

The p.t.a. was again made up as a solution of 1 gm. per ml. and standardized to a specific gravity of 1750°. Each addition was made gradually and with thorough stirring. The tube was then allowed to stand overnight, the precipitate centrifuged off and the bulk of the liquid poured into a similar centrifuge tube. The p.t.a. precipitates are of a gelatinous or spongy character which retains wort and in consequence a portion of the soluble nitrogen. In the previous series this was overcome by re-fractionation, but this tends to cause loss of material, so in the present series each tube was put into warm water in a beaker, then quickly raised to boiling, and the tube removed and cooled immediately in running water. This condensed the volume of precipitate and the liquid, after recentrifuging, was drained from the first tube into the corresponding second tube, the first tube being clamped in the draining position over the second for 10 minutes. This avoided the necessity for reprecipitation and so reduced losses which would occur from the solubility of the precipitates. Each wort underwent 19 transfers, recentrifugings and drainings and the average overall loss of nitrogen was about 2 per cent., or about 0.1 per cent. for each set of operations.

The first addition of p.t.a. to each tube was 0.15 ml., the second addition was 0.30 ml., the third 0.45 ml. and so on by amounts increased each time by 0.15 ml. to the 19th addition of 2.85 ml., which gave a total addition of 28.5 grms. p.t.a. per original 100 ml. of wort. Thus each successive addition was larger and added still further

TABLE III.
SECOND WORT AND BEER NITROGEN FRACTIONATION EXPERIMENT.
Quantities of total nitrogen are all in mgrms. per 100 ml. of original strong wort.

Fraction	Fractional additions to 500 ml.	WORT.				BEER.			
		Total Nitrogen precipitated.	T.N. as per cent. original wort N.	Amino N. per cent.	Amide N. per cent.	Total Nitrogen precipitated.	T.N. as per cent. original wort N.	Amino N. per cent.	Amide N. per cent.
A	Saturated MgSO ₄	58.2	10.8	4.9	9.5	71.8	13.4	7.2	9.2
B	+ 1.4 grms. p.t.a.	39.5	7.4	14.6	8.4	35.9	6.7	9.0	7.3
C	+ 14 grms. p.t.a.	34.5	6.4	16.5	7.3	42.9	8.0	12.6	4.9
D	+ 28 grms. p.t.a.	65.4	12.2	17.8	5.9	67.1	12.5	16.6	4.0
E	+ 56 grms. p.t.a.	38.1	7.1	28.0	4.8	16.0	3.0	28.1	10.7
F	+ 50 ml. conc. HCl.	51.0	9.5	30.0	11.5	22.6	4.2	26.2	5.4
G	"	22.9	4.3	27.1	13.8	11.8	2.2	25.7	6.7
H	"	25.5	4.8	30.6	31.7	7.2	1.3	31.0	13.3
Residual liquid	—	147.6	27.5	43.9	1.7	64.8	12.1	35.0	2.6
Original wort	—	536.5	(100)	27.2	9.6	—	—	—	—

TABLE IV.
THIRD FRACTIONATION OF NITROGEN COMPOUNDS FROM WORT BY PHOSPHOTUNGSTIC ACID.
Mgrms. of Nitrogen per original 100 ml.

WORT.	Lettering for successive precipitates.																		Sum of a to s.	Residual Nitrogen	Total Nitrogen	Recovery, per cent.	
	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r					s
	Total Phosphotungstic Acid addition per original 100 ml.																						
	0.15	0.45	0.90	1.50	2.25	3.15	4.20	5.40	6.75	8.25	9.90	11.70	13.65	15.75	18.0	20.4	22.95	25.65	28.5				
1	24.6	12.6	17.0	11.1	4.1	6.3	3.6	3.8	4.9	3.9	4.2	5.2	3.2	4.8	16.4	25.9	3.2	7.6	7.0	169	118	304	94
5	27.5	15.2	17.4	5.2	2.6	5.3	4.8	2.0	5.0	4.4	3.0	2.4	2.8	5.7	12.7	19.8	3.8	7.1	5.0	152	107	257	101
3	30.4	11.2	13.6	10.5	2.2	6.3	4.1	5.0	4.8	4.0	4.4	6.0	4.4	3.2	3.1	14.2	9.2	10.1	21.0	168	126	288	102
4	30.4	15.1	15.7	10.2	2.2	5.8	7.6	2.8	5.3	5.4	4.2	3.2	3.5	2.8	5.6	23.6	2.3	4.6	11.4	163	108	279	97
2	31.8	14.4	15.9	11.2	5.2	6.4	4.9	4.6	4.9	3.8	3.8	5.2	3.8	3.8	5.5	21.0	4.0	9.4	13.4	173	107	299	94
6	22.6	12.5	12.0	8.2	5.7	8.8	4.6	2.2	4.4	4.8	3.2	2.8	2.7	2.0	3.1	15.8	4.2	5.4	5.6	131	146	284	98
Av.	27.9	13.5	15.3	9.4	3.7	6.6	4.9	3.4	4.9	4.4	3.8	4.1	3.4	3.7	7.7	20.0	4.4	7.4	10.6	159	119	285	98

Corresponding weights of precipitate in grms.																			
1	1.22	0.47	0.92	0.56	0.42	0.36	0.55	0.49	0.56	0.33	0.40	0.53	0.33	0.55	4.15	5.53	0.70	1.69	1.78
5	1.34	0.92	1.22	0.36	0.20	0.42	0.36	0.24	0.38	0.42	0.30	0.38	0.36	1.16	3.40	4.03	0.80	1.90	1.51
3	1.22	0.42	0.62	0.33	0.18	0.35	0.34	0.48	0.55	0.38	0.50	0.84	0.54	0.35	0.36	4.17	3.53	3.20	4.22
4	1.22	0.42	0.56	0.34	0.15	0.40	0.40	0.36	0.42	0.54	0.38	0.46	0.38	0.35	1.38	6.46	0.48	0.96	2.70
2	1.34	0.36	0.78	0.42	0.32	0.46	0.52	0.32	0.46	0.29	0.45	0.52	0.37	0.46	0.94	6.11	1.41	2.04	3.01
6	1.38	0.70	0.52	0.26	0.30	0.47	0.22	0.26	0.29	0.54	0.26	0.38	0.30	0.35	0.36	2.24	1.02	1.42	1.58
Av.	1.29	0.55	0.77	0.38	0.26	0.41	0.40	0.36	0.44	0.41	0.38	0.38	0.38	0.54	1.76	4.76	1.32	1.87	2.47

Percentages of nitrogen in moist precipitates.																			
Av.	2.2	2.5	2.0	2.5	1.4	1.6	1.2	0.9	1.1	1.1	1.0	1.1	0.9	0.7	0.4	0.4	0.3	0.4	0.4

to the amount already present in the wort, so that the total concentration of p.t.a. rose regularly and rapidly and precipitated in succession compounds with a greater solubility product. Each precipitate after condensing and draining was weighed moist in the centrifuge tube, dissolved by adding dilute sodium hydroxide and transferred to a Kjeldahl flask for total nitrogen determination.

The results for amounts of nitrogen precipitated are checked by the weights of precipitate found and confirm the earlier results of fluctuating amounts of nitrogen precipitated by regular additions of phosphotungstic acid. The fluctuations found with different worts occur at corresponding points in the series of additions and the chief peaks are at 0.15 gm. p.t.a. added *per* original 100 ml. of wort and 20.4 grms. of p.t.a. Smaller ones are shown at 0.90 gm., 3-4 grms. and 26-28 grms. p.t.a. additions. With some worts the peaks, although occurring at the same p.t.a. concentration, are not so high as with other worts, indicating, it is suggested, qualitatively similar nitrogenous constituents in the worts examined, but quantitative differences.

The percentage of total nitrogen in each precipitate to total weight of moist precipitate varied in a corresponding series of jumps from 2.5 per cent. in the first precipitates through 1 per cent. in intermediate precipitates to 0.4 per cent. in the final precipitates. These results taken in conjunction with the free amino nitrogen percentages observed in the earlier and later experiments indicate, it is considered, that the compounds are precipitated in order of decreasing molecular size, since smaller molecules have less combined nitrogen attached to those nitrogenous groups which are free to link with p.t.a.

Details are given in Table IV and illustrated in Diagram II.

FOURTH EXPERIMENT

The Composition of the Nitrogen Compounds precipitated from Wort by Phosphotungstic Acid

In the work already described the total amounts of nitrogen precipitated by p.t.a. were studied in detail. Following this a further bulk of wort was precipitated, the precipitates being washed thoroughly and the nitrogen composition determined. This procedure yielded pure preparations, although

leading to some loss of total nitrogen in washing, particularly in the less complex fractions.

Wort 19, the sixth wort in the series above, was used at 1150° sp. gr. The bulk, of 1,200 ml., was divided into halves and the two sets of precipitates kept separate as duplicate checks. The amounts of p.t.a. added at each stage were chosen to correspond with the "valleys" in the curves of the precipitations found above in the previous experiment. The precipitates were washed thoroughly twice in water containing p.t.a. and redissolved by the gradual addition of potassium hydroxide. Precipitated potassium phosphotungstate (which does not adsorb nitrogen) was removed and the solution of nitrogen compounds (from 600 ml. of wort) made to 100 ml. for analysis.

The results for total nitrogen and percentage of free amino nitrogen are given in Table V. They show that the successive fractions have increasing proportions of the nitrogen as free amino groups and thus further evidence is provided that successive precipitates consist of nitrogen compounds of increasing simplicity (approximately the increase is by multiples of two). The relationship to the earlier results is shown in Diagram II.

Table V provides clear evidence for a series of jumps in composition corresponding to the breaks or "valleys" in the precipitation curve, indicating a range of discrete groups of nitrogen compounds in wort.

PREPARATION AND ANALYSIS OF COMPLEX NITROGENOUS COMPOUNDS FROM WORT

It was clearly desirable to check the quantitative work described in the earlier part of the paper by isolation of the nitrogen compounds concerned. This aspect has been studied and methods are given here for the isolation of the complex compounds.

Phosphotungstic acid itself appeared to be unsuitable as a reagent for preparative work. However, tests indicated that other acidic precipitants behave in a similar way: that is, if they are added gradually and allowed to attain equilibrium, small amounts precipitate the most complex nitrogenous compounds and subsequent increased quantities precipitate compounds of lower complexity. Acids which do not themselves contain nitrogen are suitable for this purpose, such as tannic acid and trichloroacetic acid.

TABLE V.

COMPOSITION OF NITROGEN COMPOUNDS PRECIPITATED FROM WORT BY PHOSPHOTUNGSTIC ACID.

Precipitate.	P.T.A. per 100 ml.		Nitrogen per 100 ml		Free Amino per cent. of Total N.	Suggested Designations.
	Addition.	Total.	Total.	Free Amino.		
1st	0.4 ml.	0.4 grm.	29.85 mgrms. 30.65 "	0.308 mgrm. 0.347 "	1.03 } 1.1 1.13 }	Protein.
2nd	1.7 "	2.1 grms.	22.13 " 22.00 "	0.424 " 0.405 "	1.91 } 1.9 1.84 }	Metaprotein.
3rd	3.0 "	5.1 "	19.62 " 20.15 "	0.803 " 0.903 "	4.10 } 4.3 4.48 }	Protease.
4th	8.5 "	13.6 "	11.70 " 10.60 "	0.947 " 0.852 "	8.09 } 8.1 8.03 }	Peptone.
5th	9.4 "	23.0 "	11.39 " 11.76 "	1.746 mgrms. 1.785 "	15.3 } 15.2 15.2 }	Polypeptide I.

In a number of tests tannic acid was used to precipitate the complex fractions and below are given details of its use to precipitate only the most complex fraction.

Details of Tannic Acid Method.

Twenty litres of wort at 1040° sp. gr. from a mash of a pale ale malt were pasteurized and settled bright. Five grms. of tannic acid (purified by fractional precipitation by benzene from ethyl acetate solution) were dissolved in 100 ml. of water and added gradually to the bright wort with stirring. The precipitate was removed by passing through a Sharples supercentrifuge fitted with a cellophane lining and the precipitate washed on the centrifuge. The cake of precipitate was dispersed in a small volume of water containing a little sulphur dioxide. Two volumes of alcohol were added to dissolve the protein-tannate and the solution was centrifuged. The clear solution was precipitated in two fractions (A and B) by the addition of a 1 : 1 mixture of alcohol and ether. The two fractions were each dispersed in water and lithium hydroxide solution added gradually to a p_H value of 7-8. The solutions were centrifuged and the protein reprecipitated by the alcohol-ether mixture.

The fractions gave the following results on analysis:—

Fraction.	Dry Weight.	Moisture.	Ash.	Nitrogen (Moisture and ash-free).	Free Amino, as per cent. Total N.
Tannic acid: Fraction A.	grms. 3.56	per cent. 1.5	per cent. 2.8	per cent. 12.4	per cent. 1.4
Tannic acid: Fraction B.	5.48	2.2	2.2	13.7	1.4

These preparations therefore supported the presence of the most complex protein fraction, containing about 1 per cent. of free amino nitrogen, indicated by the first precipitate with phosphotungstic acid (Table V).

Another method of preparation was also used in which advantage was taken of the fact that the nitrogen compounds can act both as acidic and as basic substances. In this the more complex nitrogenous fractions were precipitated from wort as the copper salts. These were dissolved by the addition of acetic acid and the most complex nitrogenous compounds were precipitated by trichloroacetic acid.

Details of the Dual Precipitation Method.

A mash of pale ale malt at 150° F. was pasteurized and concentrated to yield 500 ml. at 1150° sp. gr. This was dialysed in a counter-current dialyser and the non-dialysate concentrated to 500 ml. and centrifuged. To the clear liquid was added sufficient lithium hydroxide solution so that on adding immediately afterwards 20 grms. of copper acetate in 300 ml. of water the p_H was brought back to 5-6. Both solutions were added gradually with stirring. The precipitate was centrifuged on the Sharples supercentrifuge with a cellophane liner and washed. It was then dispersed in a minimum volume of water and acetic acid added to bring it into solution. The clear solution was added gradually with stirring to a solution containing 28 grms. of trichloroacetic acid. The precipitate was centrifuged off, washed with trichloroacetic acid solution and dissolved by the gradual addition of lithium hydroxide to

p_H 8. After filtration the solution was precipitated by alcohol-ether and dried *in vacuo*.

This treatment appeared to precipitate both fractions I and II of the phosphotungstic acid method as is indicated by the percentage of free amino nitrogen in the following analyses of two separate preparations:—

Sample.	Moisture.	Ash.	Nitrogen (Moisture and ash-free).	Free Amino, as per cent. Total N.
Trichloroacetic acid: Fraction A.	per cent. 6.45	per cent. 1.5	per cent. 13.6	per cent. 3.2
Trichloroacetic acid: Fraction B.	7.9	2.5	14.5	3.3

Amino-acid Composition of Preparations.

On hydrolysis with constant boiling hydrochloric acid (20 per cent.) for 48 hours the preparations gave normal results for protein analyses by the Hausmann method. For this

trichloroacetic acid preparation *B* was used, and a tannic-acid preparation (*C*) in which a larger proportion of tannic acid had been used than in that given above.

Sample.	Acid Humin.	Alkaline Humin.	Amide Nitrogen.	Non-basic Nitrogen.	Basic Nitrogen.
Trichloroacetic prep. B.	per cent. 3.8	per cent. 0.7	per cent. 12.4	per cent. 61.5	per cent. 21.5
Tannic acid prep. C.	3.3	0.7	11.5	65.0	19.6

The preparations were whitish to light-brown powders and further fractionation and purification were desirable, but this work was interrupted.

The writer gratefully acknowledges the assistance at different times in this work of Mr. W. A. Whitley, Miss V. J. Kemp, Mr. K. B. Parry and Miss B. D. Hadley.

COMMUNICATIONS

TRISTIMULUS COLORIMETRY IN THE BREWERY

By B. D. HARTONG and A. P. VAN DEN HOEK
(Phoenix Brewery, Amersfoort, The Netherlands)

INTRODUCTION

JUDGMENT of colour is a process which can be separated into three consecutive steps:

- (1) An image is formed on the retina.
- (2) The impression made on the retina is registered in the brain.
- (3) The registered impression is translated into spoken or written words.

The first two processes are given by nature and are the same for each individual having normal eyes. The third process is a mental process which must be learned. One starts learning it in early childhood and like all things which have to be learned, the ability to express in words what one sees depends on an inherited factor, mostly called intelligence, and an external factor comprising training and education. Therefore the judgment of colour will always be of a subjective kind.

Beer is a drink of which it is often said that it is drunk with the eyes before it is consumed, and certainly this is done by the

brewer who lifts his glass to the light, preferably the northern sky, and approves or disapproves of what he sees; it is difficult even for the brewer, who is an objective man of science, not to be influenced by this first impression in his final judgment of the quality of the beer under test. To the consumer the judgment of colour is much simpler. He need not express in words what he sees; he has only to reckon with his own senses giving him a stimulation, which he experiences as agreeable or disagreeable or all that lies between.

But there are two things that we know the consumer of to-day appreciates in the appearance of beer: brilliancy and cleanliness of colour. To a large extent the consumer is right in his appreciation, for both characters are correlated with the quality of beer as a whole. The task of the brewer is to achieve these qualities in his beer. But here arises at the outset the difficulty that he must explain in words or writing to his collaborators in the brewery what he sees and what

he wants to see. Of course this is already a very old problem and there are many objective laboratory methods available for measuring the colour of beer to help the brewer in his task. Most of these methods which are used as a daily routine in the laboratory use colour standards. They are made as glasses or solutions and are based on the Lovibond scale used in Great Britain and U.S.A. or the Brand scale used on the Continent. The latter is a combination of three dyestuffs giving a near approach to the colour of an iodine solution.

Since the development of photometers of all kind, the simple visual comparison against solution or glasses can be replaced by exact objective photometric measurements. To this end the photometers must be calibrated against standards. A universal method of calibration was proposed by Stone and Gray.¹ They used potassium dichromate solutions and a wave length of 440 m μ , lying close to the maximum light absorption by beer. All photometers can then be calibrated to give the result of colour measurement on the Lovibond scale. Nevertheless this improved procedure gives only the colour of beer or wort expressed in terms of concentration or intensity of colour. Also it is necessary to work with clear fluids, and if the wort or beer is hazy or cloudy it has to be cleared by filtration or adsorption or both, wherein one has to be sure that no colour is lost. Of course haziness or cloudiness of wort and beer can be measured separately by nephelometric methods. For this also there are very simple methods using visual comparison against standard solutions (*viz.* BaSO₄ suspensions), and more exact photo-electrical methods are in use. A new absolute method using no standards will be described further on in this article.

However, the intensity of the colour and the cloudiness of beer and wort do not give a complete picture of what is observed by the human eye. A third factor is the shade or tint of the colour. Here we can distinguish between greenish, reddish or greyish shades. To the consumer these shades are more or less attractive, but to the brewer they mean much more. They give him an indication of such important factors as oxidation and stability. Until lately not much attention was given to this factor, but recently a method to measure it has been proposed by Nyborg and Trolle.² They use

two narrow band filters: a blue one of 430 m μ , again near to maximum absorption by beer, but slightly different from the 440 m μ used by Stone and Gray, and an arbitrarily chosen green one of 530 m μ . From both transmission measurements two values are calculated corresponding respectively to the strength or intensity of the colour and its shade. Trolle stresses the point that the beer or wort to be measured must be absolutely clear and gives some useful instructions for achieving this.

To the normal observer all three qualities of colour: intensity, brilliancy and shade, are indivisible and present themselves as a whole and so we believed that there was room for a method measuring all three qualities together, a method imitating the observation by the normal human eye.

Such a method is known under the name of "Tristimulus Colorimetry." It found an ample application especially in U.S.A. during the last war in the field of surface colours, as of paints, enamels, papers, powders, etc., but it can also be used for luminant and transmission colours. In this last field it was applied to petroleum products, but so far we have not found any application described for the brewery. Therefore we tried this new line of approach on colour measurement in the brewery. Probably it will not lead to a method for daily routine in the laboratory, but it will broaden insight in the theoretical aspects of beer colour.

*Tristimulus Colorimetry*³.—In our introduction we wrote that judgment of colour by the individual will always be subjective. Tristimulus Colorimetry is an attempt to transform it into an objective method by making use of a statistical mean called the "Standard Observer." The 1931 *International Commission on Illumination (I.C.I.) Standard Observer for Colorimetry* embodies the average colorimetric characteristics of 17 normal observers. The method of Tristimulus colorimetry is therefore a psychophysical method, because something measured physically relates to the impression on an observer.

The name Tristimulus Colorimetry is derived from the following facts: The human retina is supposed to contain three perceptors for colour, each having a different distribution function. They have their maximum sensitivity in the blue, the green and the red region of the spectrum. The form of the

three distribution curves has been established from the experiments with 17 individual observers: the *Standard Observer*. A colour impression on the retina is composed of different amounts of the three primary stimuli. These different amounts can be measured by making use of a set-up which approximately imitates the human eye. It consists of a standard illuminant, a set of three filters and a photocell. The filters used in colorimetry have preferably a narrow band to get a good approach to a spectrophotometer, but the tristimulus filters are broad band filters corresponding to the distribution functions of the primary stimuli.

In our experiments we used a Lumetron photometer which is specially designed for tristimulus measurements. It contains two photocells which are balanced against each other with the blank solution (distilled water) and the filter in place. Then the blank is replaced by the solution to be measured and the photocells are balanced again. Thereafter one reads on a dial the percentage of transmitted light for each filter. The three values are called B, G and A; these represent respectively the blue, the green and the amber filter transmission.

The two first steps mentioned in our introduction are now fulfilled. The third step—the translation of the impression into spoken or written words—is performed by a set of equations.

The Equations.—For our purpose, which is to judge the colour of beer, the following attributes were chosen:

- (1) The hue, or tint, or shade of the colour. This means the location of the colour in the spectrum.
- (2) Saturation of the colour. This means the strength or intensity of the colour, as it is measured ordinarily with the Brand—or Lovibond—scale.
- (3) Lightness of the colour. This means brightness, luminosity, brilliancy, a sparkling colour, *etc.*

These three attributes are calculated from the measurement of a sample with three filters and describe together the impression made on the observer.

The second attribute—saturation—is the least interesting. We have it more or less in hand to make paler or darker beers and this property can be adequately described by a number from the particular scale in use in

each laboratory. The first attribute—hue—and the third—lightness—which are obtained without having to filter the beer, are much more interesting as they are related to the greenish, reddish or greyish tints. A careful study of the changes of these attributes during the different phases of brewing and during shelf-life will give a useful insight into causes and effects of these changes.

The sets of equations which have to be used to express the wanted attributes in numbers are again taken from the work of Hunter.³ We start with the values A, G and B and calculate from them the three primary stimuli. There is at once some difficulty, because the distribution function for amber has two maxima, a higher one in the red region and a lower one in the blue region. Strictly we should therefore use four instead of three filters, but a sufficient approach was obtained by Hunter with the following set of equations:

$$\begin{aligned} X &= 0.80 A + 0.18 B \\ Y &= 1.00 G \\ Z &= 1.18 B \end{aligned}$$

From these we obtain:

$$\begin{aligned} x &= X/(X + Y + Z) \\ y &= Y/(X + Y + Z) \\ z &= Z/(X + Y + Z) \end{aligned}$$

x , y and z , summing up to 1, are the trilinear co-ordinates of the I.C.I. standard system for designating colours.

We transform these values into polar co-ordinates, which prove to be suitable for beer colour designation:

$$\begin{aligned} \alpha &= \frac{2.4266 x - 1.3631 y - 0.3214}{1.000 x + 2.2633 y + 1.1054} \\ \beta &= \frac{0.5710 x + 1.2447 y - 0.5708}{1.000 x + 2.2633 y + 1.1054} \end{aligned}$$

These are rather cumbersome equations but according to Hunter they can be replaced by the following simple ones:

$$\alpha = \frac{A - G}{A + 2G + B}; \quad \beta = \frac{0.4(G - B)}{A + 2G + B}.$$

In the region of beer colours the difference between the two modes of calculation varied within 0.3 per cent., which lies well within the accuracy of the method.

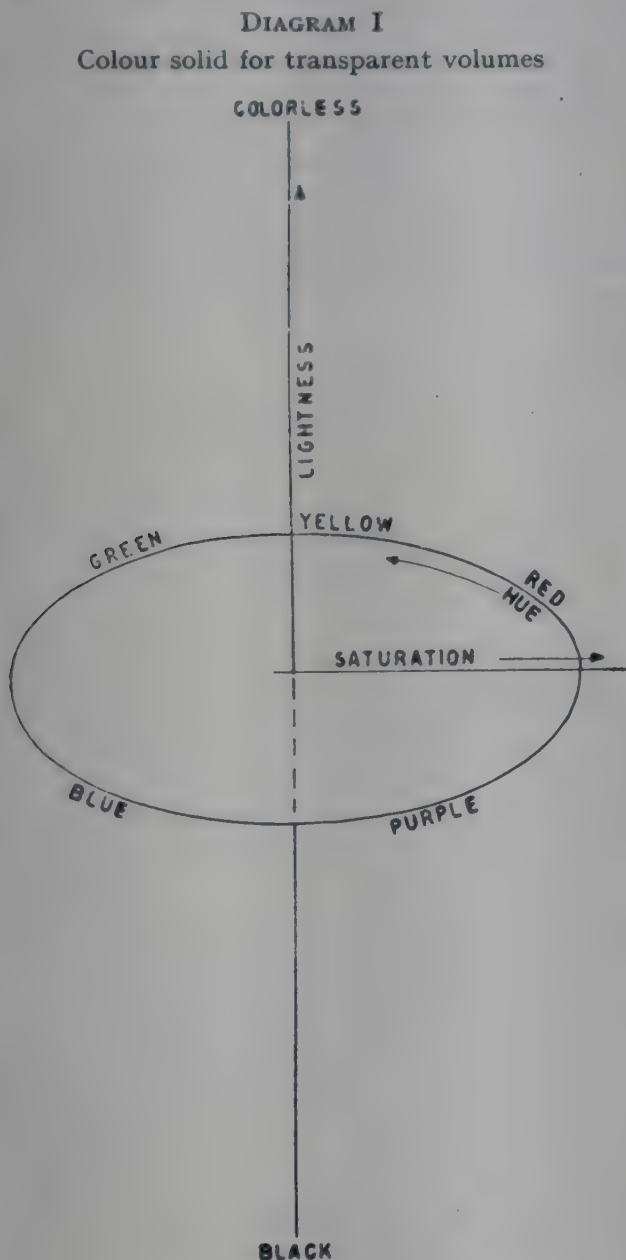
The three wanted attributes are calculated from three final equations:

- (1) Hue angle: ϕ = angle whose tangent is β/α .

(2) Saturation Index: $S = \sqrt{(\alpha^2 + \beta^2)}Y^{\frac{1}{2}}$.

(3) Lightness Index: $L = Y^{\frac{1}{2}}$.

These three attributes of colour can be represented in a three-dimensional body, called the "colour solid,"⁴ shown schematically in Diagram I.



EXPERIMENTAL

The three different attributes of colour were measured on appropriate samples, giving a complete picture of the tristimulus method for transmission colours. The instrument was the Lumetron colorimeter; the cell-diameter was 20 m.m.

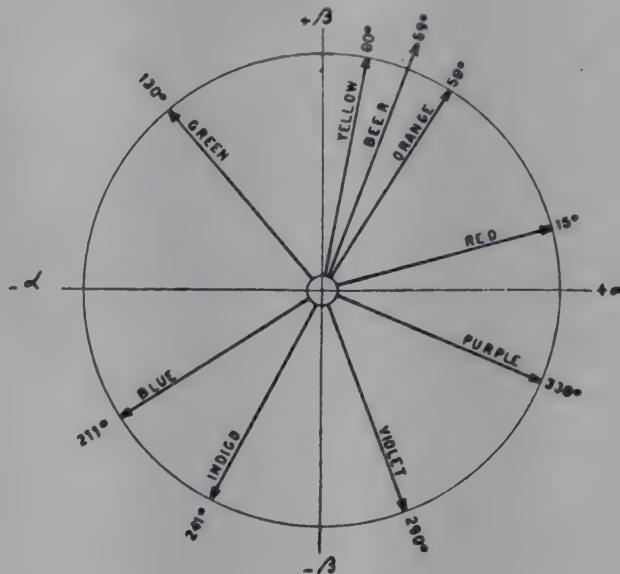
1. *Hue Angle* (see Table I and Diagram II).—The different colours fill out a disc, comprising the whole spectrum. The value

TABLE I

HUE ANGLE FOR DIFFERENT COLOURED SOLUTIONS (diluted to give suitable dial readings with the chosen layer-thickness of 20 mm.)

Colour.	Solution.	Hue angle.
Red ..	Iodeosin	15°
Orange ..	Tropeolin	58°
—	Beer	69°
Yellow ..	Potassium dichromate	80°
Green ..	Nickel sulphate	130°
Blue ..	Methylene blue	211°
Indigo ..	Indigo carmine	241°
Violet ..	Gentian violet	290°
Purple ..	Potassium permanganate	338°

DIAGRAM II
Hue diagram



for beer is found in the right place between yellow and orange. It corresponds to a spectral wave length of approx. 575 mμ, as can be taken from a diagram given by Hunter.

In a second series different beers and colour solutions were measured. They were diluted to about the same colour strength by three trained observers through visual comparison with the undiluted Export beer. The experimental and calculated data are given in Table II, and hue angles are represented in the first quadrant of the circle diagram (Diagram III).

Directing attention to the hue angle, we see that all beers and solutions lie within a sector enclosed by the potassium dichromate and tropeolin solutions. The general position of these solutions can also be seen in

TABLE II
COLOUR ATTRIBUTES OF BEERS AND COLOUR SOLUTIONS

	Light transmission.			Hue angle, °.	Satura- tion Index, S.	Light- ness Index, L.
	A.	G.	B.			
Potassium dichromate	100.0	96.6	32.5	82° 30'	0.25	9.83
Brand-solution	94.5	88.5	41.0	72° 30'	0.20	9.41
Iodine solution	96.3	91.0	49.5	72° 20'	0.16	9.54
Export beer 12 per cent. P. ..	89.3	82.9	40.8	69° 10'	0.18	9.11
Pilsner beer 11 per cent. P. ..	87.9	81.8	44.9	67° 30'	0.16	9.04
Lager beer 9 per cent. P. ..	86.4	79.5	41.2	65° 50'	0.18	8.92
Munich beer 12 per cent. P. dark ..	87.3	80.5	43.4	65° 20'	0.17	8.97
Bok beer 16 per cent. P. dark ..	86.8	79.4	41.4	64° —	0.18	8.91
Caramel solution	85.0	77.9	43.1	63° —	0.16	8.83
Tropeolin	98.8	87.5	40.6	59° —	0.20	9.35

DIAGRAM III
Hue angles in the first quadrant

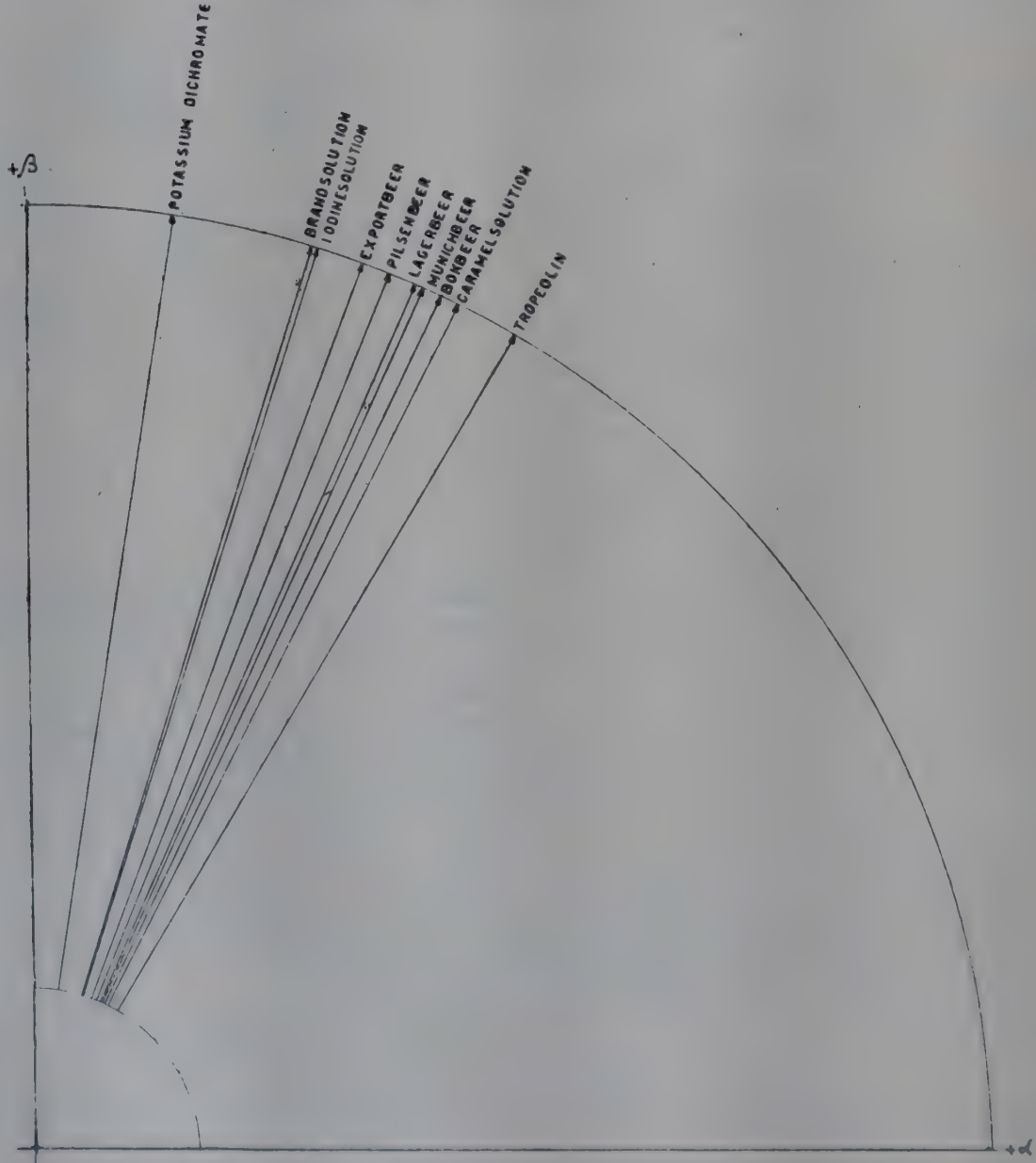


Diagram II. The Brand and iodine solutions have the same hue angle, which confirms that they perfectly duplicate one another. But potassium dichromate, and Brand and iodine solutions both to a lesser degree, show hue angles which differ from the hue angles of beer. This means that they are not very suitable to measure the colour strength of beers. Probably it will be easy to change the composition of the Brand solution a little, so as to give a hue angle of approx. 67°, lying within the beer range.

To our personal taste the Export beer has the cleanest colour. For the other beers the colour shifts to the red. They are brewed with Munich malt, coloured malt or caramel

diluted respectively to 80, 60, 40 and 20 *per cent.* of the original concentration, therefore containing always the same coloured substances. The relation of the Saturation index to the concentration is given in Diagram IV and Table III.

The values for S lie on a slightly curved line. For calibration we should prefer a straight line and maybe it would be possible to transform the equation for S, to give a straight line. The equation for S was arbitrarily chosen by Hunter, as it gave good results for surface colours. It would no doubt be permissible to make small changes for transmission colours, but we did not try it, for the intensity of the colour of beer can

TABLE III
RELATION OF SATURATION INDEX TO CONCENTRATION

	A.	G.	B.	ø.	S.	L.
Original beer 11 <i>per cent.</i> P. . .	86.5	79.2	35.6	67° 20'	0.201	8.90
Diluted to 80 <i>per cent.</i> of original	88.9	82.6	43.5	68°	0.171	9.09
60 " " " "	91.5	86.6	53.0	70°	0.137	9.31
40 " " " "	94.4	91.2	66.1	72° 20'	0.095	9.55
20 " " " "	97.5	95.5	80.9	71° 10'	0.052	9.77

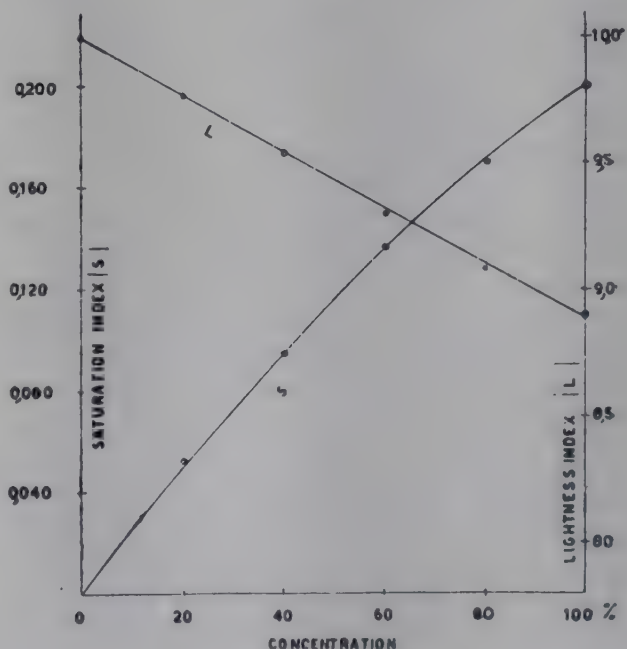
or combinations, and as we find caramel at the red side of the series this shift to the red will have something to do with caramelization. All the beers were fresh and changes caused by oxidation during shelf-life are not yet studied.

2. *Saturation (Intensity) Index.*—This attribute of colour measures the concentration of a dyestuff in a solution. In beer, which contains different coloured substances, it is better to speak of the intensity of the colour. Mathematically it is a vector in a horizontal plane of the "colour solid," Diagram I.

In Table II is found a set of Saturation Indices. The beers and solutions were diluted by visual comparison to the same intensity and the S-value should be the same. For the beers and the iodine solution this is substantially the case. Brand solution and tropeolin differ a little more but the potassium dichromate solution falls out of line. From the hue diagram we see that it is spectrally so different from the beers that the visual matching was very difficult and did not succeed too well.

To get a better insight, the saturation-index was measured on samples of beer

DIAGRAM IV
Saturation and Lightness Indices against concentration .



be measured quite simply with the help of standard glasses or solutions. Also, exact determinations with a photometer can be made with a narrow band filter of 440 mμ, as done by Gray, or 430 mμ by Trolle. For

our purpose which is the judgment of colour by tristimulus colorimetry, the saturation index gives a sufficiently proportional indication of the intensity of colour as a function of concentration.

Inspecting Table III again it will be seen that the hue angle is not constant but shows a distinct shift to the green side of the spectrum. The last two values are not very trustworthy, as the hue angle is specially influenced by the difference $A - G$. In these very diluted beers the values for A and G lie at the end of the scale where the determination is already inexact, and the difference $A - G$ may contain an inaccuracy of ± 10 per cent.

This result expressed in general terms can be described in the following way: When the intensity of the colour of beer increases it looks as if the colour shifts to the red, when the intensity decreases it looks as if the colour shifts to the green. When one inspects the range of standard solutions of the Brand scale, which are all dilutions of the same dye-stuff-mixture, one actually gets the impression that the darker ones have a redder shade. On the other hand a very pale and brilliant beer gives the impression of a greenish tinge. This phenomenon is well known in colour sensation and is called the Bezold-Brücke phenomenon.⁵ It does not apply to all parts of the spectrum. At certain wave lengths there is no shift on change of intensity, *e.g.*, yellow and blue. At others there is a shift to shorter or to longer wavelengths on dilution. In the region between yellow and orange there should be found a shift towards pure yellow, as found in our experiment. This means that the actual observation by the human eye is adequately duplicated by the tristimulus determination in the case of beer.

3. *Lightness (Brightness) Index.*—This attribute of colour is found along the vertical axis of the colour solid, Diagram I. Its definition for surface colours is quite simple, as it measures the proportion of white to black, or the grey component of colour. In transmission measurements it means the proportion of colourless to black. Of course we cannot think of transparent black colours. In our case "black" means adsorption of transmitted light by particles. These particles themselves may be white or brown or black corresponding with protein haze,

protein-tannin haze and adsorption by black colloids to be found in melanoidins. The effect to the observer is a more or less greyish tint in beer or in the reversed case a sparkling effect in beer. Therefore the Lightness index of beer complies well with the theoretical conception of the "Colour solid for transparent volumes," and its practical meaning for the judgment of beer colour is evident.

Turning back to the last column of Table II containing the Lightness index we note the following facts: (a) The highest Lightness indices (which means the most brilliant effects) are found in the solution containing potassium dichromate, iodine, Brand colour and tropeolin. We may take it that these solutions contain only molecules and no colloid particles. The maximum value for Lightness index in our scale is 10 and potassium dichromate solution has a practical ideal brightness or is perfectly brilliant and sparkling, as is easily confirmed by observation.

TABLE IV
INFLUENCE OF ADDING TANNIN TO BEER

Tannin in mgrm. per litre of beer.	ϕ	S.	L.	D.*
0	66° 30'	0.222	8.75	1.0
2	66°	0.225	8.68	4.0
4	66°	0.226	8.56	6.6
6	65° 30'	0.229	8.40	9.8
8	65°	0.231	8.27	12.2
10	64°	0.233	8.15	15.2

* Nephelometric reading

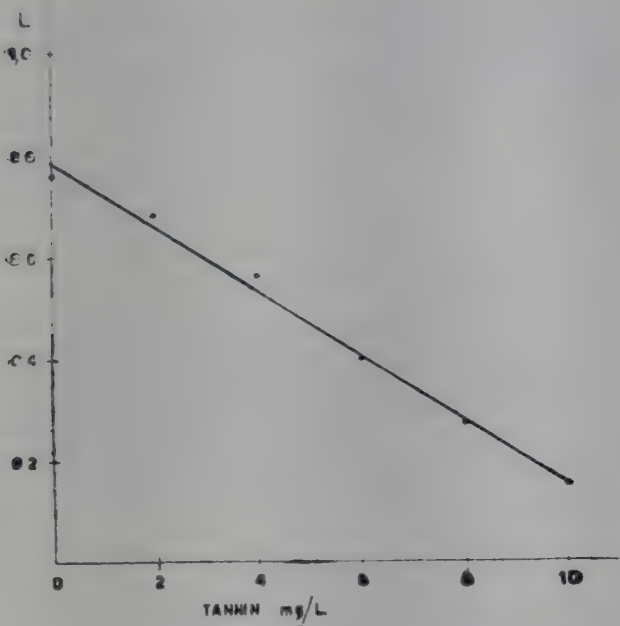
The values for the different beers lie on a lower level. They are less brilliant. Export beer has again the highest value which is not surprising as it is stabilized for tropical countries. Caramel solution has the lowest value and here we see the effect of the melanoidins containing black colloid particles.

A second impression of the significance of the Lightness index can be had from the L-values in Table III and Diagram IV. On dilution the Lightness index increases along a straight line against the concentration. This was to be expected, as we move along the vertical axis of the "colour solid." By diluting with water we change proportionally the relation colourless: black. In this case the formula of Hunter leads to perfect results.

A special experiment was made to show the influence of beer haze on the Lightness

index. To a clear beer were added increasing amounts of tannin which caused a slight haze, as in beers which begin to turn cloudy. Results appear in Table IV and Diagram V. With increasing amount of tannin, which means with increasing haziness, the Lightness

DIAGRAM V
Lightness index of beer with tannin



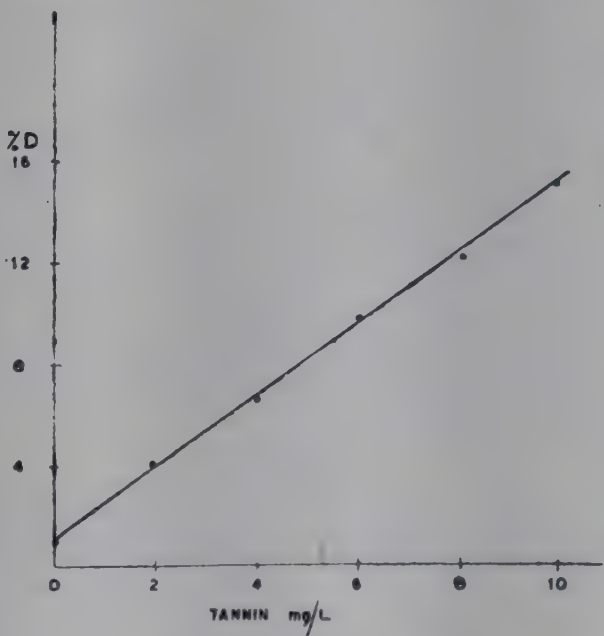
index decreases almost linearly against the tannin concentration, which confirms the formula proposed by Hunter for Lightness and applied by us to duplicate the impression made on the human observer.

At the same time we see that the hue angle decreases slightly which means a shift to red. On the other hand the saturation index shows a small increase. In comparison to the change of saturation for diluted beers as shown in Table III, we may conclude that the change of saturation on addition of tannin is too small to be observed by the human eye. Nevertheless it would have been contrary to expectation if small changes of Hue angle and Saturation index had not made themselves apparent. We have added small amounts of a coloured substance, *viz.* tannin, and the effect of it had to be found in the numerical results of the experiment.

As the Lumetron colorimeter gives the opportunity for direct nephelometric measurement without the use of a standard of comparison we put the beers of Table IV to this test. The nephelometric measurement is done in the following way: The cell with

hazy beer is placed as close to the photocell as possible. A filter is used of 580 mμ which corresponds as nearly as possible to the colour of beer. Then both photocells of the apparatus are balanced with the dial on 100. The photocell now receives the transmitted light and the maximum of scattered light produced by the haze. The cell with beer is then shifted away from the photocell as far as possible. The distance is about 12 cm. Now the photocell receives still the same amount of transmitted light but less scattered light and after balancing we get a lower reading on the dial. The difference from 100 is now a direct measure for the strength of the haze. The numerical values obtained depend on the cell diameter and the distance of displacement. These constants of the apparatus take the place of a standard of comparison. The result is shown in the last column of Table IV under D and in Diagram VI.

DIAGRAM VI
Nephelometric reading (D) of beer with tannin



Again we get a straight line. The nephelometric measurement, without the use of a prepared standard, gives a perfect characterization of beer haze. It is to be noted that even a clear beer produces a very small amount of scattered light probably caused by the colloids contained in it. Diagrams V and VI confirm one another and both give readings which are directly proportional to the strength of beer haze.

We shall further investigate which of the

two methods gives the best results, but for the purpose of judgment of colour the Lightness index must be preferred. It starts with a value 10 for an optically void fluid, decreases when the fluid contains colloids—and loses thereby already some of its brilliancy, and decreases further when the fluid becomes hazy, cloudy, and so on.

DISCUSSION

Tristimulus Colorimetry, applied to beer and expressed in the three colour attributes: hue, saturation and lightness, as parameters of the "colour solid for transparent volumes," leads to an objective judgment of the colour of beer in general. Three numbers designate what is actually seen by the normal observer "looking at a glass of beer." A large hue angle and a high Lightness (brightness) index go parallel with a clean and sparkling beer. The Saturation (intensity) index goes parallel with the depth of colour of the beer, which is of course a question of beer type and of taste.

The objectivity reached is of a psychophysical kind. The physical data obtained with the instrument are objective enough, although they certainly contain experimental errors. But the light source-filter-photocell combinations together with the set of equations try to approach the colour sensation of the human eye and no more. Here psychology comes in, which we think justified, as our consumer uses his eyes to judge the general appearance of our beer.

We hope to investigate further the changes of colour during the different phases of brewing and during shelf-life.

SUMMARY

1. The psychology of colour vision is explained. For practical judgment of colour

a psychophysical method is to be preferred. Such a method is tristimulus colorimetry. The principles of this colorimetry are laid down. It makes use of a space body called the "colour solid" with parameters: hue, saturation and lightness. These attributes of colour tell us about the shade or tint of beer, if the beer is paler or darker and if it is brilliant or sparkling, dull or hazy.

2. In the experimental part these different attributes are demonstrated with the help of coloured solutions and different beers. The results show that tristimulus colorimetry can be very well applied to beer. The designation of beer colour with three attributes gives a simple and accurate picture of the impression made on the normal observer "looking at a glass of beer."

3. A new nephelometric method for measuring haziness of beer is described. The obtained numerical expression of haziness is rectilinearly correlated with the haze produced with small amounts of tannin.

LITERATURE

1. I. Stone and P. P. Gray, *Proc. Amer. Soc. Brewing Chem.*, 1946, 40.
2. P. Nyborg and B. Trolle, *Brygmesteren*, 1948, 5, 180.
3. R. S. Hunter, *Photoelectric Tristimulus Colorimetry with three Filters*; Circular of the National Bureau of Standards, C 429.
4. D. B. Judd, *Symposium on Colour, A.S.T.M.*, 1941, p. 1.
5. D. B. Judd, *J. opt. Soc. Amer.*, 1940, IX, Bezold-Brücke Phenomenon and the hue change by admixture of achromatic light.

December, 1948.

YEAST INFECTIONS OF BOTTLED BEERS

By A. E. WILES, M.Sc.Tech., F.R.I.C.

By a suitable extension of routine biological tests, a minor yeast infection of bottled beers has been traced to its source. Although atmospheric contamination was primarily responsible for the infection, overnight development within the apparently sterile plant produced an enhanced secondary effect.

A large measure of control has been obtained by the use of very dilute solutions of sodium hypochlorite at carefully standardized concentrations.

The yeasts encountered in the investigations, although of several species, were chiefly varieties of *Saccharomyces cerevisiae*. The various strains of this species which were isolated were in no way similar to the major strains present in the brewery pitching yeast.

"THERE is no doubt that the liability of filtered beers to become hazy after some days in bottle is one of the most difficult problems which bottlers have to face." This statement, made by the late H. Lloyd Hind at Southampton in 1930¹ and requoted by Heron in 1939² remains just as true to-day as on either of these previous occasions. Whilst the wider use of pasteurization by some bottlers has done much to alleviate this type of trouble, the new range of problems arising from its introduction has discouraged its universal adoption. For the many who do not use pasteurization, biological deterioration is generally responsible for the major proportion of hazes or deposits which occur in their bottled beers and it is hoped that the present communication may assist such producers in the ultimate elimination of this kind of defect.

Hazes or deposits of biological origin are relatively easy to recognize as such in bottled beers. The majority are caused by "wild" yeasts, bacterial troubles not being encountered quite so frequently. The yeasts do not usually cause hazes but are more often deposited as a sediment, which may become quite copious if allowed to develop for a sufficient length of time. On opening the bottle with consequent disturbance of the contents, the sediment may be brought into suspension, often imparting considerable cloudiness. Whether this dispersion occurs or not will naturally depend to some extent on the powdery or flocculent nature of the yeasts present in the deposit. To confirm the presence of yeasts, if these are suspected, a portion of the beer should be subcultured in sterile wort. This test, together with

simple microscopical examination of the deposit in the bottle will usually supply the required information.

It is to be regretted that a few bottlers are unaware of the presence of such microflora in their beers. This arises from failure to keep samples from all bottlings with periodical examinations for the types of deterioration appearing. The keeping of adequate records of these storage tests is also necessary, not only for establishing the onset of trouble but also as they will enable its subsequent control to be followed more readily.

With respect to the infections themselves, there is rather a dearth of information upon this point. Lloyd Hind (*loc. cit.*) dealt more fully with non-biological hazes and Heron (*loc. cit.*) was largely concerned with the brewing of a beer which would be a more unsuitable medium for the growth of micro-organisms. Clark³ was also of the opinion that the numbers of organisms were of less importance than the type of nutriment provided.

Such factors as the quantity of carbohydrate present, percentage of alcohol, degree of attenuation, quality of nitrogenous constituents, oxidation level, air content of head space, have all been considered. These and other unmentioned factors undoubtedly have much bearing on the growth of beer micro-organisms. However, the writer's submission is that the latter method of approaching the problem can at best only partially alleviate any trouble. All beers will probably support the growth of at least some varieties of yeasts and bacteria, and variations in beer composition would probably serve only in modifying their growth rates.

It would therefore appear that successful bottling can only be achieved by the complete elimination of all biological contamination. At this point it would seem pertinent to mention that most illusory entity, "degree of commercial sterilization." Bremner and Kelly⁴ have recently commented on the use of these words and the writer fully supports their statement that they form a "deplorable phrase." Tests upon the internal portions of plant after the filtration stage and also of the beers contained therein should give completely negative results when using culture media based on wort and beer. In other words, within the limits of the media used in the tests, complete sterility should be indicated. Such results can be achieved (perhaps with difficulty) if sufficient attention is paid to plant sterilization; anything less is an unworthy target. Realization of these facts has led to the more widespread adoption of some form of biological testing in bottling stores.

The nature of the tests employed in control work will obviously vary slightly in different bottleries. Sufficient information is available on which to base an adequate routine, and selections can be made from various methods such as are outlined in the papers by Upton,⁵ Ward⁶ and Whitley.⁷ A series of such tests will then supply a measure of the sterility of the following:

- (1) The beer as delivered by the filters (of whatever type).
- (2) The bottle as mechanically washed.
- (3) The beer as delivered from the filling machines.
- (4) The particular closures used.

The testing of these points is often supplemented by further tests of such items as filter-pulp, filter-cakes, atmospheric pollution, etc.

In the bottling stores with which the writer is associated, such tests had been carried out at regular intervals for a number of years. As a result, a very comprehensive cleansing and sterilizing procedure had been evolved and satisfactory bottled beer life was generally being obtained. Occasionally during the warmer periods of the year, shortened shelf-lives were experienced because of the appearance of a typical yeast deposit. This shortened life was not serious, but it was considered desirable to know its exact

nature and origin not only in order to suppress it, but to enable immediate action to be taken in the event of a more serious outbreak of infection. A detailed investigation was therefore initiated and the findings are outlined in the subsequent sections of the present communication.

EXPERIMENTAL

Preparation of Media.

(a) Malt-wort gelatine, malt-wort agar, Gorodkows' agar, McKelvey's carrot agar, and gypsum slants, were prepared according to the methods used by Walters⁸ in his studies upon Australian beer disease yeasts.

(b) Sugar assimilation tests, and utilization of nitrate as sole source of nitrogen and of ethyl alcohol as the sole source of carbon were carried out with media prepared according to the directions of Stelling-Dekker.⁹

(c) Pasteurized-beer. Filtered beer was taken aseptically from the filter delivery sample cock and dispensed in 25 ml. quantities into 1 oz. screw-capped McCartney serum bottles which had been previously sterilized in the electric oven. The caps were well screwed down and the bottles packed in a suitable perforated metal container. The latter with its contents was then passed through a full-scale pasteurizer operating at 145° F. (20 min.). These pasteurized bottles would withstand forcing-cupboard conditions indefinitely.

Details of Routine Control Tests used.

(a) Beer Filters. The filters were fitted with sample cocks which were protected by a dome carrying a rubber sealing ring for pressure balancing. The cock was sterilized just prior to sampling by means of a spirit flame. Samples were collected aseptically in sterile half-pint screw-stoppered bottles. Most of the previously published tests utilize the plating of a 1 ml. portion of the sample on plates of malt-wort agar, subsequently incubating the plates for several days at 77° F. (25° C.). The author dislikes this method, partly because of the ease with which petri dishes can become contaminated, but more particularly because all viable yeasts are not necessarily recorded. We have therefore adopted the practice of pipetting aseptically 1 ml. of the sample into tubes containing 10 ml. of sterile malt-wort. Subsequent incubation of the tubes at 25° C.

for up to 7 days will reveal even small traces of infection.

Additional portions of the sample are also pipetted into tubes of sterile broth. The purpose of this latter test is two-fold. In the first instance we believe that when the filter is reaching the point of breakdown, the bacteria being smaller are the first to pass the filter, later to be followed by the passage of yeasts. Secondly, if water is used for rinsing the pipes before sterilization, the presence of water organisms will be detected by the broth tubes, thus indicating defective sterilization. Whilst a filter would not be rejected on results from the broth tests alone, we regard the results as valuable as a more complete picture can be obtained by their inclusion.

(b) Bottles. The usual method of testing bottles is to plug them with a sterile plug at the moment of collection. Later, 10 ml. of sterile water is introduced into the bottle. After rotation of the bottle an aliquot of the aqueous suspension of organisms (if present) is plated. Incubation of the plate then reveals the numbers of organisms present in the aliquot. For our purpose we prefer the method of Ward *et al.* (*loc. cit.*), who introduce melted sterile wort-gelatine to the bottle. Rotation of the bottle during cooling then provides a nutrient medium covering the entire surface of the bottle. The bottles are stored at room temperature for 7 days and the colonies directly counted. We have had an opportunity of using this method while a totally independent observer used a plating method on a duplicate set of bottles. The results obtained by the two methods showed a remarkable agreement. The method of Ward *et al.* has the merit of being much less time-consuming, requires less apparatus and affords a direct result.

(c) Filling machine. Samples are taken from the filling machine by substituting sterile cotton-plugged bottles for some of those bottles already on the conveyer and which are approaching the filling machine. The bottles are labelled and spaced at suitable intervals and the cotton plug removed from each at the last instant before the bottle is taken on to the machine proper. On re-emergence when full, a fresh sterile plug is inserted. By previous planning it is possible to obtain samples with the minimum of disturbance to normal bottling operations

and without duplication of samples from individual filler heads. The bottles are incubated at 77° F. for 14 days and any subsequent development of organisms is examined microscopically.

(d) Bottle closures. Normal trade bottles containing 25 ml. of malt-wort and cotton plugged are sterilized in the laboratory. In the bottling store, the bottles are placed on the conveyor leading to the crowner and the cotton plug removed at the last instant before crowning. The bottles are then incubated at 77° F. in an inverted position, and growth is recorded as being due to yeasts, bacteria or mould the mycelium of which generally grows in a submerged state.

(e) For the sake of completeness, atmospheric pollution is usually determined when carrying out the above tests. Three-inch diam. petri dishes containing 20 ml. of malt-wort agar are exposed for 5 mins. The viable falling organisms are classified as moulds, yeasts and bacteria.

Results of the Routine Control Tests.

Using the methods outlined above, the sterilizing filters were usually shown to be delivering a completely sterile product. Occasionally the broth tubes indicated infection by organisms growing in this medium. More intensive sterilization of the filters usually remedied matters in this respect.

The bottles delivered from the washing machine were exceptionally clean, counts of nil organisms were the rule, though one or two colonies were sometimes obtained. Very rare counts of 10 organisms are usually to be attributed to defective jets. The organisms encountered by us from this source have largely been moulds.

The samples from the filling machine usually showed infection, this appearing in the bottles after about 7 days in the incubator. It must be remembered that this test was carried out aerobically and was therefore rather sensitive. The aerobic nature of the tests often encouraged the growth of *Acetobacter* spp. which, however, have not been known to cause hazes in any of the bottled beers examined on these premises. Yeasts were also encountered on some occasions. When this occurred, the trade beers were usually subject to deterioration in less than 4 weeks at room temperature.

It was often noted that the beers from a few heads were sterile. That this phenomenon was not a property of the particular head was readily demonstrated by duplicating samples, when growth was often obtained from one. The phenomenon merely demonstrates the low degree of infection. This constitutes one of the grounds on which the writer bases his objection to the term "commercial sterility," for in spite of the "commercial sterility" of the beer it was capable of developing a haze of biological origin.

It was therefore evident that such infection as was occurring had its origin at some point or points between the filter delivery and full bottles as delivered by the machine. This information was somewhat limited but nevertheless supplied a sound basis upon which to base the non-routine experiments which will now be described.

Details of the Investigation.

The investigation was approached in two ways. Firstly a detailed survey was made of plant cleanliness using general bacteriological methods. Secondly, the bottles of trade beers showing a yeast deposit were examined. The yeasts were isolated in pure culture and their characteristics studied. It was hoped that the two lines of approach would dovetail as the investigation proceeded.

The long pipe-line from the filter outlet to the filling machine was broken at its various unions at the conclusion of a day's bottling. Swabs of internal portions of this pipe could then be taken. Particular attention was paid to angles, *etc.*, which might harbour "dead" pockets of beer. The swabs were transferred to sterile normal saline, rotated, and allowed to stand for half an hour. Aliquots of the saline were then pipetted into tubes of sterile wort and tubes of sterile broth. Although the broth occasionally revealed the presence of bacteria, yeasts were not detectable on any occasion from a variety of points in this pipeline.

The swab technique was also used to examine the flora of the filling tubes through which beer passes to individual bottles on the filling machine. The swabs were tested in a manner similar to that described above.

On six separate occasions swabs were taken from four different filling tubes, making a total of twenty-four separate examinations. Yeasts were isolated from only two of these examinations while bacteria growing in

broth were generally detectable. The beers obtained from the same tubes contained yeasts in four cases and acetic bacteria in twelve cases. The trade beers also from the same tubes were liable to a yeast deposit after 14 days' forcing in five samples out of the twenty-four. It was not possible to obtain any correlation between the presence of yeasts on the filling tubes and the subsequent appearance of a deposit in bottle, and therefore it followed that the filler tubes could not be considered as a major point of infection.

In the meantime, examination of the deposits from the shelf-life tests was proceeding. The yeasts in each deposit were isolated by dilution of the deposit in sterile normal saline, plating, selection of typical colonies, replating for purification and finally streaking on wort-agar slants. The yeasts isolated were then compared by the following growth tests:

- (1) Appearance of 24 hour wort culture and microscopical examination of the cells.
- (2) Appearance of colonies on wort-agar plates.
- (3) Appearance of wort-agar slant at 9 days.
- (4) Appearance of wort-gelatine slant at 10 days.
- (5) Appearance of wort culture at 2 weeks.
- (6) Growth in pasteurized beer.

Owing to the rather uncertain nature of tests (2), (3) and (4), differences in appearance were treated with some reserve but, in all, twenty different strains were isolated. The nature of these yeasts will be considered later (see chart). It became evident from the examination that (a) often two or more strains were present simultaneously; (b) the flora was changing day by day. Furthermore, the presence of bacteria of the genus *Acetobacter* was often detected, but the numbers of these were small and it was evident that their growth in bottle had been checked, probably by the more rapid growth of the yeasts and the consequent reduction in the amount of oxygen available.

It appeared evident from the varied nature of the flora that contamination could hardly arise from a pocket of infection, and additionally, as we had already eliminated the

beer filters, atmospheric contamination was probably responsible for the infection which was occurring. This source would also account for the erratic and rather light nature of the trouble.

Compressed air was being used to supply top-pressure to the filling machines and the micro-flora of the latter was examined in several ways. The first, a somewhat crude method, was to expose a petri dish (containing malt wort-agar) to the air issuing from a blow-off valve; subsequent incubation of the plates produced many colonies. A somewhat more refined method consisted in bubbling (*via* a sterile tube) the top pressure gas through nutrient media; again, contamination was readily detectable. The air lines were regularly steam-sterilized but the air-filters could not be so treated and examination of the filter material showed that this was becoming wet by moisture carried over from the compressor. It has therefore been necessary to consider a new design of air-filter which will overcome these two defects. Unfortunately one source of air ingress is unavoidable on the particular filling machines, namely the air from the bottles which passes to the top of the filler dome. On occasions when the domes have been removed for cleaning, swabs have indicated the presence of yeasts on the interior surfaces.

It was later apparent that although the atmosphere was the responsible primary source of infection, some multiplication of organisms must have been taking place within the plant. This premise was necessary to account for the relative uniformity of infection noticed on a given day. Such multiplication could hardly proceed at much more than a very slow rate during the day-time as the machines were then full of beer at low temperatures. During the night, however, conditions were rather different. The plant was flushed with nearly boiling water for half an hour; at the end of this time the residual hot water was allowed to remain in the plant overnight. The following morning the water was removed, and the plant flushed with cold filtered water before filling commenced. Samples were taken and these indicated that whereas the hot water was sterile, or apparently so, in the evening, the water obtained from the machine the following morning was not sterile, nor were the first beers run therefrom.

Accordingly it was decided to leave the

machines and pipes filled with water containing 20 to 30 parts *per* million of chlorine and to leave this solution in the plant overnight. The latter procedure was advocated by Davis¹⁰ for use in dairies where plant could not be steam sterilized and left dry. The concentrations recommended do not effect sterilization but maintain a sterile condition when this has been attained. The following morning the plant would be rinsed with filtered (sterile) water. The requisite concentration of chlorine was supplied by adding commercial sodium hypochlorite at the rate of approximately 2.5 fluid ounces to each 100 gallons of water. The strength of the hypochlorite is of course important and was determined in the usual manner by liberation of iodine from potassium iodide, followed by titration with decinormal sodium thiosulphate. The final concentration of chlorine in parts *per* million was readily checked by means of the *orthotolidine* reagent,¹¹ matching the colours against standards prepared from potassium dichromate and copper sulphate solutions. The actual test is carried out as follows:—

25 ml. of the sample (or other suitable quantity) are diluted to 1 litre (to give a chlorine content of less than 1 p.p.m.). To 100 ml. of this solution contained in a Nessler cylinder is added 1 ml. of the *orthotolidine* reagent which is prepared by dissolving 1 gm. of *orthotolidine* (m.p. 129° C.) in one litre of dilute hydrochloric acid (100 ml. of concentrated acid made up to 1 litre with distilled water). The Nessler tube and contents are set aside for 5 minutes to allow the colour to develop. The colours developed may be compared with those produced by suitable dilutions of sodium hypochlorite solution or, alternatively, permanent standards may be prepared from copper sulphate and potassium dichromate solutions. As the quantities of the latter do not vary proportionately with the chlorine it is necessary to consult the original literature for the appropriate tables.

Using the above procedure it was found that if the mains, filling machines, *etc.*, were filled each evening with the water containing 20 p.p.m. of available chlorine, by the following morning it was still possible to detect from 10 to 15 p.p.m.; a greater loss of chlorine than this 5 to 10 p.p.m. would most likely be accounted for by a quantity of organic matter within the plant. In

Chart Showing the Cultural Characteristics

Reference.	Growth in malt wort, 24 hr. at 25° C.	Growth in wort 3 days at 25° C.	Growth in wort 15 days at 25° C.	Appearance of cells in malt wort agar slant—3 days at 25° C.	Appearance of wort agar slant culture 6 weeks at 15° C.	Giant colonies wort-gelatine 40 days at 15° C.
A	Cells mainly oval to pear-shaped 7-8 μ $\times$ 3-5 μ , chiefly with small spherical bud at broad end, singly and pairs.	Cells as at 24 hr. Singly and pairs, occasional threes. A deposit.	Slight ring, no film. Deposit.	Small oval 4 μ $\times$ 2 μ to large oval 10 μ $\times$ 5 μ and narrow cells 8 μ $\times$ 3 μ — 20 μ $\times$ 4 μ , singly and occasional pairs.	Nearly flat, buff, smooth, soft, moist, glistening. Edge slightly serrate.	Flat, matte, smooth, pale buff, edge slightly lobate, radial lines at periphery.
B	Cells largely (9-10) $\times$ (4-5) μ ; pear shaped in pairs, both cells of almost equal size.	Cells mainly rather large oval to pear shaped 12-15 $\times$ 8 μ , singly. A flocculent deposit.	Weak broken film. Deposit.	Small to large elliptical (5-20) $\times$ (3-5) μ with some elongated cells, singly and pairs.	Flat, smooth, buff, flaky, slightly irregular, moist glistening surface. Edge entire.	Circular, flat, smooth. One concentric ring. Outer zone darker grey inner zone cream.
C	Growth slow, cells round to oval (8-9) $\times$ (6-7) μ .	Cells small, sausage shaped and pear shaped 8-9 μ $\times$ 5 μ . A powdery deposit.	Thick ring. Slight film. Deposit.	Oval to nearly round (5-12) $\times$ (4-8) μ , singly and pairs.	Nearly flat. Centre grey, outer buff, moist, glistening. Edge lobate.	Flat, matte, smooth, buff, many radial lines, lobate edge.
D	Cells long-narrow, chiefly 12 μ $\times$ 3 μ and oval 4-7 μ $\times$ 3-5 μ ; singly, pairs and threes	Cells oval to pear shaped, rather variable. A powdery deposit.	Slight ring, no film. Deposit.	Small oval 5 $\times$ 2-5 μ to large oval 11 μ $\times$ 5-6 μ . Singly, but chiefly long narrow cells 6 μ $\times$ 2-5 μ to 30 μ $\times$ 4 μ . Singly and pairs.	Nearly flat, grey-buff, surface moist, soft, glistening, finely dotted giving sparkling appearance. Edge serrate.	Centre raised and pitted, matte, buff radial lines at periphery.
E	Cells oval to pear shaped (6-15) $\times$ (3-4) μ . Singly and pairs.	Cells as at 24 hrs. A powdery deposit.	Very slight broken ring. Deposit.	Small oval 4 μ $\times$ 2 μ to large oval 10 μ $\times$ 6 μ , occasional enlarged cells 15 μ $\times$ 3 μ .	Buff coloured matte-glistening, soft, wrinkled. Edge lobate.	Flat matte, smooth buff. Edge lobate, centre raised and wrinkled. Radial lines.
F	Film only, cells long oval 7-8 μ $\times$ 4 μ in chains.	Cells as at 24 hrs. Films thicker. Deposit.	Thick film. Deposit.	Not tested.	Not tested.	Not tested.
G	Cells elongated. $\approx$ 5 μ $\times$ 2 μ in chains.	As at 24 hrs.	Thick film. Deposit.	Not tested.	Not tested.	Not tested.
H	Cells nearly spherical (5-6) $\times$ (4-5) μ mainly in pairs and fours.		Broken film. Ring. Deposit.	Very small oval 3 μ $\times$ 1-5 μ some very long and narrow 30 μ $\times$ 2 μ . Singly.	Very shiny, flat, pale buff, spreading easily, soft edge, lobate.	Centre raised and white matte. Outer translucent cream. Edge lobate.

using chlorine solutions for this purpose it must of course be emphasized that higher concentrations should not be allowed prolonged contact with metal pipes; hence the necessity for a reliable method of determination of the quantity of chlorine.

The use of such weak chlorine solutions was put into effect during a period of warm weather when a certain amount of infection was being encountered. The results were quite striking. Samples of beer in bottle were maintained at forcing temperatures (80° F.) for over 5 weeks without the appearance of any haze or deposit. The occurrence of several similar periods of higher night temperatures without undue spoilage of the non-pasteurized bottled beer samples on

long storage have led us to conclude that the system, though by no means the answer to all infection problems has given us a considerably increased control over them in our bottling stores.

Nature of the Yeasts Infecting Bottled Beers.

In the interests of completeness it was decided to attempt some classification of the yeasts isolated from the bottled beers. During the investigation outlined above it was not possible to pursue this study and the yeasts were maintained in 10 per cent. sucrose solution. Unfortunately three cultures were not viable when re-examined, so that the data for these is incomplete. The yeasts were examined by the standard

The Yeast Varieties Isolated

Spore formation.	Sugar fermentations.						Nitrate as sole source of nitrogen.	Ethyl alcohol as sole source of carbon.	Probable identity and remarks.
	Glu- cose.	Suc- rose.	Mal- tose.	Galac- tose.	Lac- tose.	Raff- inose.			
Spores on gypsum slant at 7 days and on Gorodkawas' agar at 4 days. Spores round and smooth.	+	+	+	+	-	‡	No growth.	Scanty growth.	<i>Saccharomyces cerevisiae</i> (Variety A). In all six different strains of this variety were isolated. All grew readily in pasteurized beer producing either powdery or flocculent deposits.
Spores on Mc Kelvey's agar at 7 days; spores without copulation, round and smooth.	+	+	+	+	-	‡	No growth.	Scanty growth.	<i>Saccharomyces cerevisiae</i> (Variety B). Two strains isolated. Differ only in appearance of wort-agar slant.
Spores on McKelvey's agar after 6 weeks and gypsum after 7 days, without copulation, round and smooth.	+	+	+	+	-	‡	No growth.	Scanty growth.	<i>Saccharomyces cerevisiae</i> (Variety C). Two strains isolated. Both grew well in pasteurized beer.
Readily on Gorodkawas' agar and gypsum 7 days. No copulation. Round and smooth.	+	+	+	+	-	All.	No growth.	Scanty growth.	Many of the cells in young wort and wort-agar cultures are rather longer than is the case with <i>S. carlsbergensis</i> . The cells do however resemble descriptions of <i>S. uvarum</i> , a somewhat similar species.
Sporing freely on Gorodkawas' agar at 7 days. No copulation. Round and smooth.	+	+	+	+		All.	No growth.	Scanty growth.	<i>Saccharomyces carlsbergensis</i> . Two strains isolated.
No spores found.		No	fermentations.				No growth.	Fair growth.	Probably a <i>Mycoderma</i> sp., but to be certain many more attempts would have to be made to induce sporulation in order to eliminate <i>Pichia</i> and <i>Hansenula</i> as possibilities.
			Not tested					Not tested.	Unfortunately this culture was lost before spore formation was sought. It is therefore equally likely that it was a species of <i>Mycoderma</i> , <i>Pichia</i> or <i>Hansenula</i> .
No spores found.	+	+	+	+		‡	No growth.	No growth.	Although spore formation was not detected, only three spore-forming media were tried. It does seem quite likely that the species is a member of the asporogenous group forming pseudomycelium (wort-agar slant 3 days).

methods laid down by Stelling-Dekker (*loc. cit.*) and Lodder,¹² and their characteristics are shown in the accompanying Table.

It will readily be seen that the great majority of the infecting yeasts were strains of various varieties of the species *Saccharomyces cerevisiae*, two strains belonged to the species *S. carlsbergensis*, and the remainder were typical brewery air yeasts, two of the film forming type (one being *Mycoderma* sp.) and the remaining ones probably yeast-like fungi with pseudomycelium.

The members of the species *S. cerevisiae* are of some interest. Some are closely allied to strains isolated in brewery forcing-tray tests and which appear also to grow fairly

well in draught beer. They bear no resemblance to the major strain of typical Yorkshire stone square yeast used for pitching in this locality. The latter strain is characterized by the cells (at 24 hr. in malt wort at 77° F.) being round (5-10 μ) and forming clusters of united cells called "*Sprossverbände*" by Stelling-Dekker.

The species of yeast growing in the bottled beers are therefore not of any outstanding differences from those one would normally expect to find in draught beers and it is therefore quite likely that they originally entered the bottling stores on the wide variety of equipment, both from the brewery and the trade.

SUMMARY

1. The greatest contributory factor in bottled-beer infections is probably the organism itself; alterations in beer composition can hardly result in total elimination of infection.

2. Biological control tests form a sound basis on which to base a logical investigation as to the actual source of the organisms. An example of such an investigation has been outlined.

3. The infection was shown to arise primarily from the atmosphere, secondary development probably taking place within the plant overnight. By the use of very dilute chlorine (hypochlorite) solutions, it was possible to obtain a good measure of control over the infection.

4. The organisms responsible for the infections were mainly varieties of *Saccharomyces cerevisiae*, but were not related to the typical pitching yeast strain. The ability to grow quite readily in beer was a feature common to all the yeasts isolated.

Acknowledgements.

In conclusion, the author wishes to thank Miss I. Mellor and Mr. G. C. Brown for assistance with the experimental work,

Dr. T. K. Walker for valuable criticism, and the Directors of Messrs. Tennant Bros. for permission to publish the results of the investigations.

REFERENCES

1. H. Lloyd Hind, *this Journ.*, 1931, 15.
2. H. Heron, *ibid.*, 1939, 434.
3. S. T. Clark, *ibid.*, 1939, 430.
4. T. S. Bremner and H. E. Kelly, *ibid.*, 1948, 258.
5. A. W. H. Upton, *ibid.*, 1939, 390.
6. T. J. Ward *et al.*, *ibid.*, 1939, 387.
7. W. A. Whitley, *ibid.*, 1948, 21.
8. L. S. Walters, *ibid.*, 1943, 245.
9. N. M. Stelling-Dekker, *Die Hefesammlung des Centralbureau voor Schimmelcultures*, I Teil. *Die sporogenen Hefen*. Amsterdam: Verhandel. Akad. Weten., 1931.
10. J. G. Davis, *Proc. Roy. Sanitary Inst.*, Health Congress, Harrogate, 1948, 148.
11. Water Sterilization. I.C.I. Publication.
12. J. Lodder, *Die Hefesammlung des Centralbureau voor Schimmelcultures*, II Teil, 1ste Halfte. *Die anaskosporogenen Hefen*. Amsterdam: Verhandel. Akad. Weten., 1932.

The Laboratory,
Exchange Brewery,
Sheffield, 3.
December, 1948.

SACCHAROMYCES CARLSBERGENSIS AS A CAUSE OF TURBIDITY IN DRAUGHT BEERS

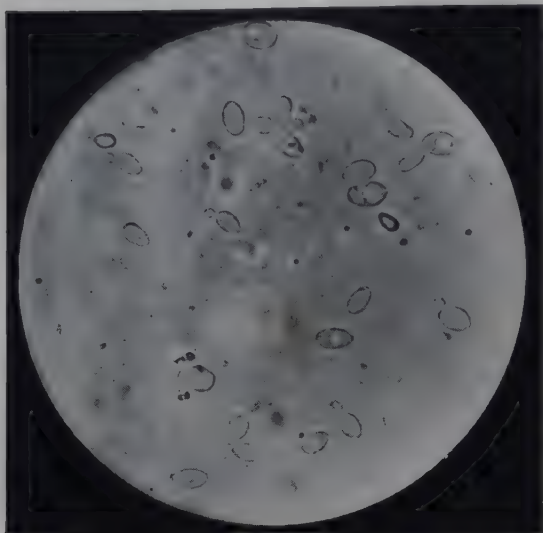
By A. E. WILES, M.Sc.Tech., F.R.I.C.

THE paucity of information on British "wild" yeasts has been commented upon from time to time by writers in this *Journal* and more recently by B. M. Brown in *Chemistry and Industry*.¹ That more records have not appeared may well be due to the difficulty of identification which formerly existed in this field. Often it was assumed, albeit without good reason, that Hansen's classical yeasts were also responsible for the majority of sick frets and turbidities arising in English beers. The taxonomic chaos amongst the yeasts was considerably cleared by the publications of Stelling-Dekker² and Lodder³ but the greater opportunities thereby afforded for more easy identification still remain very much neglected.

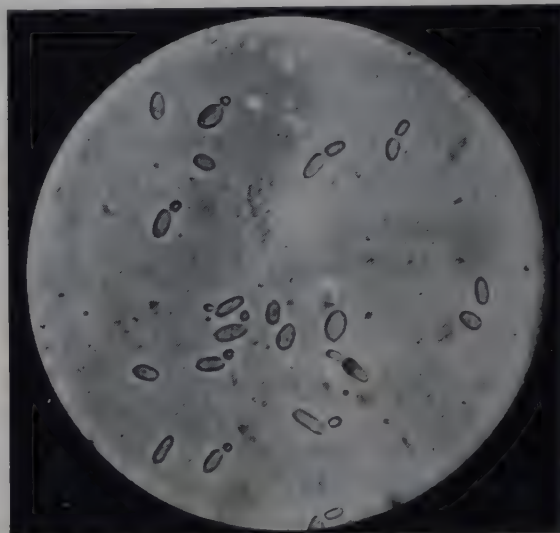
The "wild" yeasts are probably encountered quite frequently in brewery laboratories, and the author is of the opinion that these laboratories could make at least some small contribution to our meagre knowledge in this field until such time as the Institute of Brewing can investigate the matter more fully and correlate the little information already published. It is for these reasons that the observations described below are recorded.

During the Summer of 1948, widespread haziness of draught beers occurred throughout the country. Insofar as this company was concerned, the trouble commenced during late April and increased in magnitude throughout the months of May and June,

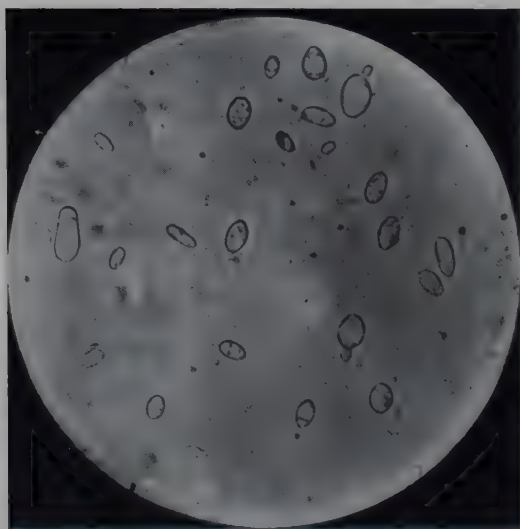
Photomicrographs of *S. carlsbergensis*, Yorkshire Haze Strain I



1. Cells from 24 hr. wort culture
at 80° F. ($\times 450$).



2. Cells from 7½ hr. wort culture
at 80° F. ($\times 450$).



3. Cells from 72 hr. wort-agar slant
culture at 80° F. ($\times 450$).

rising to a maximum in July, August and September. Although the beers were brilliant on receipt at licensed premises (after the normal time for settling), the haze often appeared within a very short period thereafter, rendering the beer quite unsaleable, though only on visual grounds. Only a small percentage of the total output was affected, but the continued occurrence of the trouble in spite of all technical action was a source of considerable anxiety to all concerned.

Samples received in the laboratory showed that the hazy beers always contained a considerable number of yeast-cells in suspension and, as shown in the table, the turbidity of the sample bore a direct relationship to the number of cells in suspension. Removal of the cells by filtration afforded a brilliant filtrate. It was therefore concluded that the development of the cells was the factor responsible for causing the turbidity.

TABLE

RELATIONSHIP BETWEEN THE NUMBER OF YEAST CELLS IN SUSPENSION AND THE HAZINESS OF THE BEER

Sample No.	Cells in suspension, per ml.	Haze units.
1	1.00×10^6	80
2	2.89×10^6	95
3	4.19×10^6	150
4	6.19×10^6	220
5	6.91×10^6	300
6	8.00×10^6	310

Note.—The haze units, measured photoelectrically, are expressed in terms of parts per million of Fullers' earth BPC required to produce an equivalent turbidity when suspended in a medium of the same Lovibond tint.

Samples of the turbid beer examined in wort-agar plate culture showed the presence of a mixed flora, but this always contained a certain number of colonies which after 12 days' growth were approximately 7 mm. in diameter, buff coloured, and of a smooth moist-glistening appearance with an entire edge. Selection of typical colonies led to the isolation of the yeast described below. When the yeast had been fully examined, further samples of turbid beer were plated and shown to contain from 30 to 100 per cent. of this particular yeast, expressed as a percentage of the total cell-count.

The yeast was shown to grow rapidly in beer at temperatures as low as 54° F. (12° C.), producing a powdery type of deposit dispersing very readily, and it was undoubtedly the cause of the majority of the turbidities experienced with the draught beers of this company during the Summer of 1948. As shown subsequently, the yeast was identified as a strain of *Saccharomyces carlsbergensis* which for the reasons to be enumerated we have designated as Yorkshire Haze Strain I. This strain was also isolated by us from the beers of at least five other breweries within a radius of 60 miles, and to that extent it may perhaps be considered to be a somewhat widespread cause of trouble. We have also isolated from local beers two other somewhat related organisms but as yet have no direct evidence that these can be held responsible for similar haziness.

Materials and Methods.—With one exception the media were all prepared according to the methods of Stelling-Dekker (*loc. cit.*). These have also been described in this *Journal* by Walters.⁴ For the determination of raffinose fermentation Stelling-Dekker used the Kluyver-Iterson fermentation tube. As the latter could not be obtained, we originally determined the residual melibiose by a volumetric method but later abandoned this in favour of a direct melibiose fermentation. The melibiose broth was prepared by the fermentation of raffinose-yeast-water by a strain of *Saccharomyces cerevisiae* according to the directions of C. E. Skinner.⁵ The broth was then used for fermentation tests in the usual manner using the Durham tube method.

Cultural Characteristics.

1. Growth in malt-wort—24 hrs. at 77° F. (25° C.):
Fermenting, hazy, deposit.
Cells in above (5–12) $\mu \times$ (3–4) μ , oval to elongated.
Singly and in pairs.
2. Growth in malt-wort—3 days at 25° C.:
A deposit, cells as above.
3. Growth in malt-wort—14 days at 25° C.:
Deposit only.
4. Growth in malt-wort—1 month at 59–68° F. (15–20° C.):
Deposit only. Supernatant liquors brilliant.

5. Growth on wort-agar slant—3 days at 25° C.:
Buff coloured, moist glistening.
Cells in above (4–15) $\mu \times$ (2–8) μ oval and elongated, mainly singly, some pairs.
6. Appearance of wort-agar slant—2 months at 15–20° C.:
Pale grey-yellow, nearly flat, soft, moist glistening surface dotted, sometimes wrinkled, edge jagged.
7. Giant colonies on wort-gelatine—1 month at 15–20° C.:
Circular, edge slightly wavy, cream matte, flat, centre slightly pointed. Faint radial lines becoming more marked towards edge.
8. Sugar Fermentations.
Glucose +; Maltose +; Sucrose +; Galactose +; Lactose –; Raffinose +; Melibiose +.
9. Spore formation.
Spores formed readily on Gorodkows' agar in 3 to 4 days; on gypsum in 1 week and on wort-agar slants after 2 weeks at room temperature.
Spores formed without copulation.
10. Spores round and smooth, one to four *per* ascus; diameter of spores approx. 3 μ .
11. Utilization of ethyl alcohol as sole source of carbon: scanty growth.
12. Utilization of nitrate as sole source of nitrogen: negative.

Taxonomic Considerations.—It will be apparent from the above description that the organism is a member of the genus *Sacch-*

aromyces in the group of melibiose-fermenting yeasts, namely : *S. carlsbergensis*, *S. pastorianus*, *S. validus*, *S. logos* and *S. uvarum*. The size and shape of the cells in young wort and wort-agar culture specify it as *S. carlsbergensis*. Stelling-Dekker (*loc. cit.*) lists five varieties of *S. carlsbergensis*, but the strain described above differs somewhat from each of these in some particular. It is not our wish to create a new variety without excellent reason, but at the same time the strain is of some economic importance to the brewing industry and therefore requires some distinctive designation. We have accordingly named the described strain as *Saccharomyces carlsbergensis* Yorkshire Haze Strain I.

Cultures of the organism described will gladly be forwarded to any interested person, institution or company.

In conclusion, the author wishes to express his thanks to the Directors of Messrs. Tennant Brothers, Ltd., for permission to publish the above communication.

REFERENCES

1. B. M. Brown, *Chem. & Ind.*, 1947, 609.
2. N. M. Stelling-Dekker, *Die Hefesammlung des Centralbureau voor Schimmelcultures*. I Teil. *Die sporogenen Hefen*. Amsterdam: Verhandel. Akad. Weten., 1931.
3. J. Lodder, *Die Hefesammlung des Centralbureau voor Schimmelcultures*. II Teil. *Die anaskosporogenen Hefen*. Amsterdam: Verhandel. Akad. Weten., 1934.
4. L. S. Walters, *this Journ.*, 1943, 245.
5. C. E. Skinner, *J. Bact.*, 1947, 53, 47.

The Laboratory,
Exchange Brewery,
Sheffield, 3.
December, 1948.

BITTER PRINCIPLES OF THE HOP

By F. GOVAERT, D.Sc.

(Director of the Organic Chemistry Laboratories, University of Ghent, Belgium)

HOPS play an important part in the processes of brewing; the variety and amount used determine to some considerable extent the class and quality of the finished beer. The importance of the bitter substances of hops in brewing is indicated by the fact that for more than a century they have been the subject of many studies. Although their chemical composition has been elucidated, the scientific basis of the hopping process is still a matter for discussion and further investigation.

In the last few years, the author and his assistants, in collaboration with the *A. van der Stricht Foundation*, have undertaken an exhaustive study of these bitter substances, and the results of some of these investigations are the subject matter of the present communication. The primary aim in these investigations was the preparation from hops of pure humulon and lupulon. We were able to obtain these two substances in a state of absolute purity by application of the chromatographic technique.

The starting point is an extract of fine-ground hops in methyl alcohol. The filtered extract is acidified with dilute sulphuric acid and extracted with light petroleum. On evaporation of the solvent from this second extract, the bitter acids and soft resins are obtained. This residue is then dissolved in benzene and passed through a chromatographic column containing silica gel as adsorbent. The α - and β - acids pass through the column and all resinous impurities are adsorbed and retained by the silica gel. The benzene solution may be evaporated, the bitter acids again treated with methyl alcohol and subsequently separated and purified.

The term "soft resins" has been used above to indicate those substances which accompany the bitter acids in the extract and which can be separated therefrom by chromatography. This is not in accordance with previous conceptions. In fact, it has been customary to include in the term "resins" not only the products of oxidation and polymerization of the bitter acids but also the acids themselves. The combined

α - and β - fractions, as determined by the older methods of analysis, do indeed constitute the soft resins. But the terminology is faulty since humulon and lupulon are not resinous in character. Rather are they substances which can be obtained in crystalline form and of which the chemical constitution is known. Furthermore, we have been able to demonstrate that the resins which accompany the bitter acids, and which we determined in addition to lupulon in the β - fraction, have no brewing value (bitter value) whatever.

Thus, while preparing the α - and β - acids, we have been able to isolate chromatographically large amounts of soft resins and have determined their bittering power. As a result we have found that the resins separated quantitatively from the α - and β - acids do not communicate any bitterness to the beer even when quantities at the rate of 900 grms. of hops to the hectolitre are added (2 lb. to 22 gallons approx.).

It would seem, therefore, that the resins which are formed in the hop at the expense of the α - or β - acids do not yield, in the wort-boiling process, any substances which contribute bitterness to the beer. It may be concluded, then, that humulon and lupulon are the only specific constituents of the hop giving rise to characteristic bitterness; and of the two, humulon has an incomparably greater bittering power than lupulon.

This indispensable character, however, is not imparted to beer by the humulon or lupulon molecule as such but, in respect of humulon at least, this substance undergoes during the brewing process a molecular transformation which results in the formation of an isomeric substance which we have called *iso-humulon*. We have prepared this substance in a state of purity, have determined its chemical composition and regard it as absolutely indispensable in the brewing process. Up till now it has not been possible to detect even traces of the α - or β - acids in beer, but in dealing with very large quantities of beer we have recently been able to isolate different substances related to the bitter constituents. One of these has been identified

as *iso*-humulon. Compared with humulon this isomer is much more soluble and bitter.

With regard to the β - acid, we have been able to show in numerous laboratory brews that the action of a certain amount of lupulon in addition to the α - acid during wort-boiling, though favourable, is exceedingly small. It is thus possible that the β - acid also produces interesting products of transformation, but in trifling amounts. Researches on this aspect are still proceeding.

Summing up the above considerations, we may clearly draw the conclusion that it is the content of pure bitter acids, and especially that of the α - acid, quite separate from the resins, which is the indispensable criterion in determining the brewing value of the hop. It therefore becomes necessary to evaluate them in an absolute quantitative manner.

As the chromatographic method supplies a means of quantitative separation of the bitter acids from the resins, their analysis will thereby be greatly simplified. The method of determination elaborated below depends upon this preliminary chromatographic separation of the extract from a known weight of hops. The methyl alcohol solution containing the total α - and β - acids is treated with lead acetate and the α - acid is determined as the insoluble lead salt of humulon. The β -acid is determined by potentiometric titration.

A new method, also based on preliminary chromatographic separation, utilizes the relatively high optical activity of humulon, which is estimated polarimetrically. The

total acids are determined by potentiometric titration and the lupulon is calculated from the two results. The new method occupies only 2-3 hours and gives results, as in the first method, which are absolutely reproducible. The method may be readily applied in the brewing laboratory, and enables the brewer to work at any time with hops of which the content of active bitter acids is accurately known.*

Thanks to this rapid and quantitative determination of the bitter acids, the problem of improving hop varieties can now be faced with greater confidence. Indeed, it is now possible to perform an analysis of green hops in a regular and systematic manner and obtain reliable results in a minimum of time.

The formation and evolution of bitter acids during maturation of the hop may thus be studied. By determination of the α - and β - acids before and after drying, the causes of losses, so frequently deplored in this operation, may be investigated. A study on these lines has been inaugurated this year in collaboration with the *Institut Belge du Houblon*. The results will shortly be published in *Fermentatio*.

REFERENCES

- Fermentatio*, 1926, No. 1, 1.
Fermentatio, 1926, No. 8, 33.

* Professor R. Baetslé, Director of the *Institut Supérieur des Fermentations de Gand*, reports that his staff now employ the simple and rapid method described above and carry out daily a large number of analyses on behalf of the brewing industry.

ABSTRACTS

I.—MATERIALS AND ANALYSIS

Influence of Growth-Regulating Chemicals on the Malting of Barley and the Composition of Malt. A. D. DICKSON, H. L. SHANDS, and B. A. BURKHART (*Cereal Chem.*, 1949, 26, 13-23). This work was carried out on Oderbrucker and Wisconsin Barblless barleys grown in 1938 and 1943. In most of the experiments the growth-regulating chemicals were added to the steep water, and all the barleys were steeped at 16° C. (61° F.) to 45 per cent. moisture and malted for six days at this temperature and moisture content. The results of the experiments are in general agreement with those obtained by previous workers. When very dilute solutions (0.02 to 10 p.p.m.) of indole-acetic acid and naphthalene-propionic acid were used, there was no apparent effect on germination or composition of the malt. Concentrations of 2.5 to 10 p.p.m. of naphthalene-acetic acid and naphthalene-acetamide were also without effect, but concentrations of 50, 100 and 200 p.p.m. began to produce changes. Rootlet development was retarded, although there was small change in the length of the acrospire, and with naphthalene-acetic acid at 200 p.p.m. concentration the malting loss with the Oderbrucker barley was reduced by about 2 per cent. The extract, diastatic power, α -amylase, and wort nitrogen/total nitrogen ratio of the malt were also slightly reduced by this treatment. The lower concentrations showed less effect, and Wisconsin Barblless was less influenced than Oderbrucker by the treatments. Similar treatment with naphthalene-acetamide caused slight increases in the extract and wort nitrogen/total nitrogen ratio of the malt, and the effects of this substance on rootlet development and malting loss were similar, but less than those produced by the acid. Rough measures of respiration of the growing barley showed either no effect or a slight stimulation by both acid and amide. Three esters of naphthalene-acetic acid that have been used to prolong the dormancy of stored shrubs and tubers were applied in vapour form to steeped barley. No marked effect on the development of the amylases was observed, but the higher concentrations of two of the esters caused an increase in the wort nitrogen/total nitrogen ratio of the malt. The practical malting implications of these findings are discussed.

A. A. D. C.

Effect of Sodium Chloride in the Extraction of Malt at 20° and 30° C. on Alpha-Amylase and Diastatic Power Values. W. J. OLSON, M. T. LOWRY and A. D. DICKSON (*Proc. Amer. Soc. Brewing Chem.*, 1948, 13-19),

Optimal extraction of α -amylase from malt is not obtainable with distilled water. Use of dilute salt solutions extracts additional amylase and protects the enzyme in solution. Determinations have been made of the quantitative effects of sodium chloride at 20° and 30° C. on α -amylase extraction, on diastatic power and on the ratio of the 20 and 30° C. α -amylase values. The 32 malts used were prepared in an experimental maltings to give optimal production of amylase and maximum retention of activity. The barleys were steeped to 48 per cent. moisture, germinated 6 days at 16° C. and 48 per cent. moisture, dried and cleaned. 15 gm. samples of the ground malts were extracted at 20° C. with 300 ml. water, or 0.5 per cent. sodium chloride, for 2½ hours with frequent shaking and filtered. Extractions at 30° C. were similar, but were carried out for 1 hour. 0.5 per cent. sodium chloride gave the maximum extraction obtainable with this salt and this concentration had no measurable effect on dextrinization time. Diastatic powers were determined at 20° C. by the method of the American Association of Cereal Chemists. α -amylase dextrinizations were made at 20 and 30° C. by modifications of the methods of Handstedt *et al.* (this *Journ.*, 1940, 52) and Olsen *et al.* (this *Journ.*, 1945, 144).

Use of 0.5 per cent. saline instead of water increased the recovery of α -amylase at 20° C. by 25.1 to 68.7 per cent. and at 30° C. by 9.1 to 44.3 per cent. Whilst the ratio of the 20 and 30° C. α -amylase values was reduced by use of saline, the degree of variability of this ratio between malts was of the same magnitude whether saline or water was used for extraction, so that a precise temperature conversion factor cannot be obtained by use of 0.5 per cent. saline. The factors contributing to the differential effect of salt in α -amylase extraction are unknown. A small increase of D.P. accompanies saline extraction.

C. R.

Comparison of Ficin and Papain in the Estimation of the Beta-amylase of Barley.

R. F. BAWDEN and W. G. ARTIS (*Proc. Amer. Soc. Brewing Chem.*, 1948, 1-10). In barley, β -amylase exists partly free, partly combined. A comparison has been made between commercial preparations of papain and ficin (a proteolytic enzyme from the fig plant) as agents for the liberation of β -amylase from barley. 5 gm. portions of ground barley were extracted with 100 ml. distilled water containing amounts of ficin or papain varying between 1-20 per cent. of the weight of the barley. Extraction temperatures of 20, 30, 40, 45 and 50° C. for

extraction periods of up to 6 hours were employed: for periods of over 6 hours, temperatures of 20 and 30° C. were used. β -amylase was evaluated by the A.S.B.C. procedure (this *Journ.*, 1945, 262). Soluble protein in the barley extracts was determined by Kjeldahl's method. All enzyme preparations used were free from β -amylase.

An interrelationship exists between extraction time, extraction temperature and protease concentration. As the concentration of either enzyme acting for 2½ hours at 20° C. increases from 1 to 20 *per cent.*, β -amylase activity of the extracts increases. There is a limit of extraction temperature beyond which recorded β -amylase decreases, this decrease being accentuated by increase of extraction time, especially with ficin at temperatures above 30° C. The decrease is attributed to heat inactivation and adsorption of β -amylase. The highest β -amylase values are given by 2 *per cent.* ficin and 10 *per cent.* papain. Comparable values are given by 6 hours at 40° C. and 22 hours at 20° C. No consistent relationship exists between protein solubilized and β -amylase found, but under favourable conditions of time and temperature increase of soluble protein runs parallel with increase of β -amylase. The effect of grind on β -amylase extraction is pronounced, but follows no definite trend and small particle size does not necessarily mean better extraction. There appears to be an optimum p_H for extraction, characteristic of the enzyme used, but some differences may be due to barley variety. Differences between different enzyme preparations employed under optimal conditions are small.

C. R.

Chromatographic Analysis. M. DESCAMPS (*Bull. Assoc. anc. Etud., Louvain*, 1948, **44**, 125-144, 171-190). Chromatographic analysis is based on the fact that the chemically closely related compounds of a mixture may be adsorbed from solution to different extents by a particular adsorbent. The adsorbent, a finely-powdered solid, is well packed into a glass tube plugged at the lower end, and the solution to be analyzed is poured on and drawn through under moderate suction. The differential adsorption causes the components to be adsorbed in a succession of layers down the column (visible if the components are coloured), and the separation of these layers is increased and sharpened by washing the column with either some of the liquid used as solvent or some other solvent. The column of adsorbent is then removed intact from the tube, the individual layers separated by cutting, and the adsorbed substance eluted from each by triturating the solid with the suitable solvent and filtering. In addition to separating the components of a mixture the procedure can be used for testing whether a

product is homogeneous or not, for checking the identity of two products, for removing impurities, and for getting a substance which is in dilute solution into more concentrated form. The author describes in considerable detail the types of apparatus available, and follows this with some general remarks about adsorbents. In principle, any finely-powdered substance will serve, provided it is insoluble in the solvents and eluants used and does not react with these liquids or with the substance to be examined. The adsorbing capacity depends on the mode of preparation, activation, size of particles, solvent used, the presence of impurities, and possibly other as yet unknown factors. Most adsorbents can be regenerated if necessary by prolonged extraction with alcohol, followed by drying and heating. Many organic adsorbents can be reclaimed by recrystallization. Rate of movement of the solvent through the column may be increased by mixing a filter-aid with the adsorbent or by using a mixture of large and small particles of the adsorbent. For the analysis of a very complex mixture, different adsorbents may be used in successive layers. Choice of a suitable adsorbent is a matter of empirical trial, although experience will be a guide. The methods of preparation, specifications and qualities of alumina, magnesia, lime, chalk, magnesium carbonate, talc, silicate clays, fuller's earths, carbon and cane sugar as adsorbents are outlined. Choice of solvent depends on the solubility of the mixture and the solubility and activity of the adsorbent. There is no sharp boundary between solvents and eluants; what will serve as solvent for some substances can often be used as eluant for less strongly adsorbed substances. In general, elution is possible by using the original solvent to which has been added 1 to 2 *per cent.* of methyl or ethyl alcohol. The author lists a number of solvents, distinguishes briefly between their several uses, and describes means for their essential purification. In his description of the detailed procedure to be followed in preparing a chromatogram (the column containing the adsorbed components of a mixture, with the layers separated by "development" with the solvent), he stresses the importance of having a homogeneous, well-packed column, of not allowing the column to run dry at any time between adding the solution and completing the development, and of avoiding too strong a suction, which will tend to draw the centre of each separated layer below its periphery. A micro-chromatographic method is described, whereby the most suitable conditions and materials for analyzing a given mixture may be decided. When using water as a solvent an important factor is the p_H . This can be as low as required, but should never be greater than 11. Development is usually carried out with water

at a p_H slightly higher than was used as solvent, and elution with water more alkaline still (p_H 10 to 11), or with an aqueous solution of pyridine. The author illustrates a section of his paper devoted to the relation between adsorption and chemical constitution by reference to the carotenoids, and describes several ways of indicating zones of separation in a column when the separated substances are colourless.

A. A. D. C.

Determination of Tryptophan and Lysine in Microvolumes of Protein Hydrolyzates.

R. MILLARES and S. G. DAVIS (*Analyst. Chem.*, 1949, **21**, 414-416). A microbiological procedure is described in which conventional media are used, but total volumes of only 2 c.c. of combined hydrolyzate and medium are needed for each assay tube. The basal media were those described by Reisen *et al.* (*J. Biol. Chem.*, 1946, **165**, 347). Samples for the determination of lysine were hydrolyzed by autoclaving with 2 N hydrochloric acid for 6 hours at 121° C. (250° F.). For determination of tryptophan, samples were hydrolyzed with pancreatin and hot mucosa as described by Greenhut *et al.* (*ibid.*, 1946, **165**, 325). One grm. samples of fresh tissue, or 100-200 mgm. samples of protein, were used, and the hydrolyzates diluted to bring them within the ranges of the assay curves. Duplicate tubes at 0.2, 0.4 and 0.6 c.c. levels were set up, diluted with water to 1 c.c., 1 c.c. of medium added, and the tubes sterilized at 121° C. The tubes were then inoculated with 0.02 c.c. of the standard micro-organism suspension (*Lactobacillus arabinosus* 17-5 for tryptophan and *Leuconostoc mesenteroides* P-60 for lysine), incubated at 37° C. (98.6° F.) for 72 hours, and the response of the micro-organisms measured by titration of acid produced with sodium hydroxide (0.01 N for lysine, 0.05 N for tryptophan). For the standard curves, tubes containing 0, 0.1, 0.2, 0.3, 0.4, 0.5 (each in triplicate), 0.6, 0.8 and 1.0 c.c. (each in duplicate) of the standard amino acid solutions were set up (5 microgrm. *per* c.c. of *l*-tryptophan, 20 microgrm. *per* c.c. of *l*-lysine), and treated as above. The reliability of the method has been checked by simultaneous determinations using conventional methods on various protein hydrolyzates. Close agreement between values was obtained.

A. A. D. C.

Determination of γ -Isomer of Benzene Hexachloride.

N. R. TRENNER, R. W. WALKER, B. ARISON and R. P. BUHS (*Analyt. Chem.*, 1949, **21**, 285-290). The method described is based upon the addition of a known amount of γ -hexadeuterobenzene hexachloride to a known amount of the sample to be assayed, isolation of some of the mixed (isotopically) γ -isomers, and determination of the ratios of the

isomers by direct infra-red spectrophotometry. Directions are given for the preparation of the tracer compound, using deuterium oxide and a nickel catalyst at 200° to 300° C., and for the isolation in pure form of the γ -isomers from the sample. The spectrophotometric procedure is also described in detail. The most accurate results are given by measuring the fraction of the protio-isomer in the isolated specimen, and it is convenient and sufficiently accurate if, in the isolation, the last traces of α -isomer are not separated, but a correction of -0.5 unit made on the fraction of γ -isomer found in the sample. The introduction of deuterium into the molecule causes extensive change in its infra-red spectrum, and the specific use of infra-red spectrophotometry in determining the mass isotope dilution, as described here, is believed to be original. Since impurities are without effect on the accuracy of the method, its precision is such as to make it a standard by which the reliability of other methods of assay may be judged.

A. A. D. C.

Spectrographic Determination of Tin and Aluminium in Beer.

O. R. ALEXANDER and V. M. BISKE (*Proc. Amer. Soc. Brewing Chem.*, 1948, 69-75). Investigations on chill haze in beer, which has been attributed to polyvalent metal ions, especially tin (this *Journ.*, 1942, 51), necessitates a rapid means of determining tin in beers. The spectrographic method described serves also to determine aluminium. For spectrographic analysis, beer must be provided with a matrix of constant composition to serve as internal standard. Accordingly, lead nitrate as a spectroscopic buffer, and bismuth and titanium chlorides as internal standard elements for tin and aluminium respectively, are added in relatively large quantities. Concentrated ammonia is added dropwise with stirring to p_H 8.5-9.0, and after boiling 5 min. and allowing to settle, the precipitate is filtered off, washed, and dried at 100° C. The precipitate is transferred to an agate mortar and crushed to a fine powder. Both test samples and standards are prepared in this way, the latter consisting of tin and aluminium-free beers to which amounts of standard solutions have been added to cover a range of 0 to 0.5 *p.p.m.* of tin and 0 to 10 *p.p.m.* of aluminium. Electrodes of $\frac{1}{4}$ -in. diameter graphite are coated with a thin deposit of the prepared precipitate, using metal-free rubber cement as adhesive. A pair of electrodes for analysis consists of one prepared and one unprepared, but otherwise identical, electrode. Examination is carried out using a 2 mm. spark gap, a current of 3 amp. at 2,500 volts and an exposure time of 60 sec., spectra being recorded on 35 mm. film. The determinations of tin are carried out using the tin line at 3175.0 Å and the bismuth line at 2993.3 Å and those of

aluminium using the aluminium line at 3082.2 Å and the titanium line at 2956.6 Å. The intensity ratios (Sn 3175.0 : Bi 2993.3 and Al 3082.2 : Ti 2956.6) of the standards are plotted against the corresponding concentrations of tin on logarithmic paper and a smooth curve drawn. The values of tin or aluminium in test samples corresponding to observed intensity ratios can then be read off from this curve. C. R.

II.—FERMENTATION

Improved Techniques in the Propagation and Shipment of Pure Yeasts. S. LAUFER and H. MOHR (*Proc. Amer. Soc. Brewing Chem.*, 1948, 58-62). Single-cell strains of yeasts are isolated by plate culture and grown up in tubes. For the propagation of larger cultures, Pasteur flasks are used as the next stage. The vessels recommended are round, Pyrex flasks of more than 2 litre capacity, with flat bottoms, and fitted with two narrow outlet tubes—a plugged one at the top and a second at a 30° angle with this tube, closed with rubber tubing and a glass rod, which is used for inoculation. The Pasteur flask contains 1,200 ml. sterilized wort. When its contents are fermenting vigorously, they are transferred through the side tube to Carlsberg vessels. The latter have evolved from the original tin-lined copper vessels, through aluminium, to the new glass vessels, which eliminate difficulties of cleaning and the danger of copper poisoning. They consist of 12 litre Florence flasks (wort capacity 7-8 litres) fitted with two side arms. The upper arm is set 4 in. from, and at a 30° angle to, the neck and serves for inoculation and addition of more medium. The lower arm extends horizontally 2 in. from the bottom and serves for draining off spent medium, leaving the yeast as a sediment on the bottom. The vessel is supported in a stainless-steel wire basket. Liquid yeast from the Carlsberg vessel is transferred aseptically, with the aid of pressure or vacuum, to a 2 litre yeast shipping container, which is totally enclosed except for two outlets at the top controlled by needle valves. These containers, and also modern pure culture apparatus, are made in stainless steel as compared with their old counterparts in tinned copper. C. R.

Effect of High-frequency Electric Fields upon some Micro-organisms. K. S. LION and B. S. GOULD (*Amer. Brewer*, Jan., 1949, Part 1, 21-24). The authors have investigated the effect of heat induced in yeast and moulds by placing them in high-frequency electric fields. Cultures of *Saccharomyces cerevisiae* were suspended in water, sprayed on to filter paper and allowed to dry. The sheets were stretched across the interior of flat polystyrene containers above and beneath which were the condenser plates of a high-frequency generator. After submitting the

yeast specimens to the action of an electric field of 200 volts *per sq. cm.* and a frequency of 25 megacycles for variable times, the filter papers were cut up and transferred to tubes of sterile malt extract, the number of yeast cells in suspension being determined at intervals. With the shorter exposures up to 86 seconds, growth of the yeast was greater than in the controls and 20 seconds exposure yielded an increase of 37 *per cent.* in a series of experiments. Beyond 150 seconds the number of viable cells progressively diminished. Similar experiments carried out with surface liquid cultures of *Penicillium* sp. showed that the yield of penicillin was increased with short periods of exposure to the high-frequency radiation, but longer exposure induced retarded growth of the mould and an inhibition of the production of the antibiotic. Different species of *Penicillium* responded in a different degree to the same amount of exposure to the electronic fields. These results may serve to explain some of the anomalous results obtained in the electronic pasteurization of beer. T. J. W.

Production of Food Yeast from Wood Hydrolyzates. E. E. HARRIS, M. L. HANNAN and R. R. MARQUARDT (*Industr. Engng. Chem.*, 1948, 40, 2068-2072). This report describes experiments made to determine the amount of nitrogen, phosphate, potash, magnesium and sulphite that must be added to wood-sugar solution to get maximum yeast yield; the amount of air required, and the effects of adding oxygen to the air, of increasing the depth of the propagator, of sugar concentration, and of wood species upon the growth of the yeast. The wood hydrolyzates contained 4.5 to 6 *per cent.* of reducing material (hexoses, pentoses, non-sugars), calculated as glucose, about 0.1 *per cent.* of calcium sulphate, 0.5 to 1 *per cent.* of other salts and about 1 *per cent.* of other organic matter. Fermentation was carried out by a continuous process at a p_H maintained between 5 and 5.5, and the yeast used (except in one experiment) was a strain of *Torula utilis*. Maximum yeast yields were obtained with 3.2 lb. of nitrogen, 1.5 lb. of phosphorus pentoxide and 1 lb. of potassium chloride *per* 100 lb. of reducing sugar; the addition of magnesium sulphate did not change the yield or rate of growth of the yeast. Solutions to which had been added 3.4 lb. of nitrogen as urea, 1.6 lb. of phosphorus pentoxide as phosphates, and 1.1 lb. of potassium chloride for each 100 lb. of reducing sugar gave yeast yields of 40 to 50 *per cent.* of the fermentable sugar in a throughput time of 3 to 4 hours, the propagator having a mechanical foam controller to increase the contact of air with the solution. The presence of sulphite during the neutralization of the sugar prior to yeast production and

the addition of oxygen to the air supply, both increased the yield of yeast. Increasing the depth of the propagator gave slightly higher efficiency in the utilization of the air and, in the equipment used, sugar in concentrations of over 6 per cent. was not efficiently utilized. This work was done on Douglas fir hydrolyzate, and it was found that bakers' yeast could be propagated on this solution with good sugar utilization and yeast yield. Bakers' yeast would not grow in oak hydrolyzate, but *T. utilis* showed no great differences in behaviour in hydrolyzates from larch, Douglas fir, lodgepole pine and red oak. Yield of yeast was highest from oak and lodgepole pine, but utilization of reducing substances was best in the fir and pine. A. A. D. C.

Grain Alcohol Fermentations: Submerged Mould Amylase as Saccharifying Agent. E. H. LEMENSE, V. E. SOHNS, J. CORMAN, R. H. BLOM, J. M. VAN LANEN and A. F. LANGLYKKE (*Industr. Engng. Chem.*, 1949, 41, 100-103). A description is given of the production of alcohol by the use of mould enzymes (instead of malt) for conversion of the starch in a large pilot plant using the results obtained in small-scale experiments as a basis. A culture of *Aspergillus niger* NRRL 337 was transferred to successively larger amounts of sterile medium containing concentrated distillery stillage, glucose and chalk in water, each cultivation being stirred and aerated during the submerged growth of the mould. A 10-gallon culture so prepared was transferred to 535 gallons of a mash (see below) in a seed culture tank and left to grow for 24 hours. The mash consisted of a mixture of ground maize, chalk and thin stillage, and after being heated to gelatinize the starch it was sterilized at 274° F. under pressure, cooled to 86° F. and in part used as above and the rest (17,500 gallons) transferred to a closed fermenter to which the 24-hour mould culture was also added. This was agitated and aerated for 60 hours with air that had been sterilized by compression and passage through a column packed with activated carbon, and the saccharified mash was run into the distillery for fermentation. The advantages of the above method are stated to be the greater resistance of the mould enzymes to inactivation by acid than the amylases of malt and reduced cost of operation since the enzymic activity of the mould costs less than that of malt, and in a distillery using 5,000 bushels of grain daily the resulting economy would amount to about £120 per day. T. J. W.

III.—BEER

Some Aspects of Beer Foam. K. BECKER (*Brewers' Digest*, 1949, 24, No. 1, 37-41, No. 2, 43-46). Apart from its aesthetic value, the

formation of a good persistent head on beer in a glass is an indication of good all-round quality of the beverage. The formation of a desirable foam depends upon a number of factors, including surface tension, viscosity and vapour tension of the liquid, the nature of the gas in solution, temperature, humidity, the character and cleanliness of the vessel containing the liquid, etc. The persistence of foam largely depends upon the presence of substances in solution which tend to concentrate at the surface of a liquid and reduce the surface tension at that level. When beer is poured or drawn the reduction of pressure causes the formation of minute bubbles of carbon dioxide which during their upward movements adsorb surface-active substances such as hop resins, proteins, higher alcohols, organic acids, etc., and carry them to the surface. Thus a fine elastic film is formed around each bubble which persists until, owing to certain changes, the film collapses, allowing several small bubbles to coalesce into larger ones which exhibit less stability, and the foam begins to break down. Although foam formation and stability are independent characteristics, the two overlap at any period during the life of the foam. Beer foam is of a unique type and differs considerably from the heads formed by various detergents and by certain edible substances sometimes used to improve beer head, but these yield a foam of artificial character, the appearance of which is not appreciated. Owing to the solubility of carbon dioxide, bubbles containing this gas only are liable to break down rapidly, but in dispensing or pouring beer air is entrapped, and owing to its lower solubility tends to render the head stable. Many attempts have been made by modifying the brewing process to produce good heads on the finished beer, but little success has been achieved in this way for foam formation depends upon a delicate balance of activities which is readily upset. The addition of certain gums may produce a head of natural appearance, but this necessitates that the beer should contain at least four times its volume of gas to produce the foam.

Although little is definitely known about the precise effect of malting and brewing procedures upon head formation, the accumulated experience of many observers enables some deductions to be made. Relatively high malt kilning temperatures favour head production and stability without adverse effects upon enzymic activity, extract or colour. Excessive protein modification during mashing is liable to inhibit foaminess owing probably to the destruction of the molecules of medium complexity on which head formation largely depends. Hop resins contribute to a considerable extent to foam formation and the recent necessity for the curtailment of hop rates has had some undesirable effects in this connection. Sometimes a change of yeast

will effect an improvement in beer head, but no definite conclusions can be drawn from the results obtained by rapid fermentations or those proceeding more slowly. Foam formation may be adversely affected by inadequate as well as by over-filtration. The addition of various substances to beer or modifications in the brewing process which may have advantageous results in one brewery or one type of beer, may have the opposite effect in another or may yield an indifferent result. A common cause of unsatisfactory beer head is the presence of minute traces of oil or grease. These may be derived from the lubricated bearings of the plant, contaminated bottle brushes, or the air supply, the latter acquiring oil from the compressors or pockets of grease in the pipe lines. The oil may be eliminated by passing the compressed air through a column of activated carbon of 10 to 30 mesh, such a filter requiring renewal every 6 to 18 months. Several methods have been adopted for evaluating beer foam, those devised by Blom and by Helm being widely used, although somewhat complicated. Much may be learned about the head by pouring the beer by hand into a glass in the usual way, and this will provide additional information which would not be indicated by the more scientific methods.

T. J. W.

Influences of Certain Variables in Taste Testing as Applied to Beer. R. R. SLATER and G. K. FRENKEL (*Proc. Amer. Soc. Brewing Chem.*, 1948, 106-113). Tests were carried out to show the influence on the results of taste tests when certain variables were either controlled or not controlled. The variables chosen for investigation were the volume of sample taken into the mouth for tasting and the time interval between tasting two samples for comparison. The basic method adopted was that of paired comparisons. 30 subjects of no special "ability" were chosen for the tests. Samples were presented in 1-oz. whisky glasses at a temperature of 17° C., the subject being screened from viewing any of the preparative work involved. Each subject rinsed his mouth with water, was then given a taste-sample as a warm-up and again rinsed his mouth with water. He was told he would receive 3 pairs of samples, that the pairs might or might not be different and was asked to state a preference or "no preference." No question of difference between samples was raised. Each glass was filled with 10 ml. just prior to being given to the subject, who was instructed to take the entire 10 ml. into the mouth at once. 30 sec. after swallowing the first member of the first pair, he was given the second member and asked to state his preference and to swill out his mouth. 60 sec. after swallowing the second member, the first member of the second pair was presented and

so on until three paired comparisons had been completed. No subject took part in a second sitting until at least three hours later and he was not allowed to take part until at least one hour after having eaten. Beers chosen for test were of the same colour. When the first controlled test was completed, a second similar trial was carried out, after which the uncontrolled trials were begun. For the latter test, conditions and samples were the same as before, but 25-35 ml. of sample was given, both members of a pair were presented together and the subject was allowed to taste in any way he pleased except that the first member of a pair had to be tasted first. The uncontrolled trial was repeated a second time. The results of these tests were examined statistically and it is concluded that the controlled method offers the better probability of correctly ranking different beer samples on the basis of preference. A further test was carried out in which the subject was asked to judge whether the members of a pair were the same or different and, if the latter, whether he had any preference and if the preference was definite or not. This trial indicated that it would be advantageous first to determine if a subject detected a difference between members of a pair and then to ask for a preference.

C. R.

Clarification of Beer. R. LANEAU. (*Bull. Assoc. anc. Etud., Louvain*, 1948, 44, 153-170). This is a discussion of the comparative merits of four systems of beer clarification by: (1) the pulp filter, (2) the plate filter, (3) the centrifuge, and (4) filtration with kieselguhr, based on a series of papers which have recently been read on the subject. Clarification has three aspects: its influence on the quality of the beer, its capacity to give a bright beer, and its cost and ease of operation. No clarifying system will improve the flavour of a good beer, but a filter will tend to remove undesirable flavours, to an extent depending on its adsorptive power. The centrifuge has least effect on flavour. No system of clarification, properly conducted, imparts any particular odour or flavour to beer. Considered solely in its capacity to give a bright beer, the best clarifying system will be the one that has maximum powers of retaining micro-organisms and haze-producing matter without lowering the resistance of the beer to changes of a biological or non-biological nature. The most brilliant beers are obtained from plate or pulp filters. A kieselguhr filter, if carefully managed, will give as good results, but is more often used as a pre-filter. A beer clarified by centrifuge will satisfy the standards of the public, although it will not have the polish of filtered beers. The greatest biological stability is attained with the centrifuge and the plate filter, but difficulty in making plates to an invariable standard of

efficiency lessens their reliability. Centrifuged beers are slightly more subject to chill haze than filtered beers, and of these the plate-filtered beer is the least susceptible. From the point of view of ease of operation the centrifuge comes first, but for output the kieselguhr filter takes the lead and the centrifuge comes last. As a general rule the pulp or kieselguhr filter is suited to the needs of the large brewery, and the plate filter or centrifuge to the needs of the small brewery. Loss of beer inherent in the process is greatest with the pulp filter and least with the centrifuge. Comparison of costs is difficult, as they are influenced so much by circumstances. A fundamental difference between the systems is that clarification by centrifuge is constant, whilst clarification by filter is variable, although with careful supervision and choice of agent the kieselguhr filter gives results closely approaching constancy. The author suggests that the use of two systems in conjunction may in some instances give the best results. A.A.D.C.

IV.—PLANT, MACHINERY AND BY-PRODUCTS

Concentration of Detergent in Bottle Soakers. W. NEKOLA (*Brewers Digest*, 1948, No. 11, 45-46). Investigations carried on for a week by determining the concentration of alkali in samples of the detergent solution from a bottle-soaking tank taken at different levels showed marked discrepancies. At one period during the test the values obtained were 9.0 per cent. at the bottom of the liquid and 4.2 per cent. at the surface. The detergent was added in the form of solid caustic soda or as a sludge of this mixed with a small amount of water, and a 3 per cent. solution was required for use. Actually a much larger amount than would yield this concentration had been added. Such discrepancies and wastage would be avoided by adding the detergent as a 10 per cent. solution followed by thorough mixing by mechanical agitation or by the injection of steam or compressed air into the bottom of the tank. This should be carried out whenever the tank is charged or when "make up" or water are added. T. J. W.

Recent Development in Bottlewashing.

J. C. L. RESUGGAN (*Bottling*, Jan., 1949, 38-42). The author describes the recent application of quaternary ammonium compounds to the washing of beer bottles and emphasizes their advantages over the usual alkaline detergents. Six grades of detergent for the above purpose have been developed by a London company and these contain a quaternary compound together with alkaline carbonates. Since many waters are hard, scale-preventing preparations such as Calgon are often added to eliminate deposits, but many

quaternary compounds are incompatible with these substances. This has however been overcome in the case of dioctyl dimethylammonium chloride and thus scale prevention and water softening may be combined with the wetting and bactericidal action of the quaternary compound, and the solution acts as an efficient lubricating agent for the moving parts of the machine. These compounds destroy yeasts as well as bacteria and with the very low concentrations used the washed bottles are free from any odour, flavour or alkalinity. The low alkalinity of these preparations ensures the removal of all kinds of labels entire and with very slight pulping, and the loss of strength of the solution during use is stated to be less than when caustic alkalis are employed, whilst foaming is reduced to a minimum.

T. J. W.

Ion Exchange in Water Treatment. H.B. GUSTAFSON (*Industr. Engng. Chem.*, 1949, 41, 464-466). For industrial use it is usually sufficient to remove only part of the dissolved salts from water, although by means of the newer resinous ion exchangers the whole of the mineral matter may be eliminated if necessary. Until 1930 the production of inorganic base-exchange materials was actively developed, their chief use being for softening water. At the present time much attention is being devoted to the manufacture and use of the more versatile resinous ion exchange preparations. Although the latter may be made more specific in their actions, it is probable that sodium aluminium silicate will be more in demand for a long time on account of its efficiency and cheapness. Both domestic and industrial demands are mainly for the softening of water by the sodium-cycle exchange materials, but the relative costs for treatment of municipal supplies based upon a unit of 1,000 gallons having 20 degrees of hardness are approximately 10s. for home rental service, 4s. for a home-owned softener and from 3d. to 1s. for treatment at the waterworks. The choice of exchange material for any particular purpose is dependent upon the temperature of the water to be treated, its p_H value, the desirability of avoiding the taking up of silica and the nature of the substances to be removed from the water. Most frequently a raw water to be used for boiler purposes which is alkaline from the presence of bicarbonates of calcium and magnesium is best treated by chemical precipitation with lime and soda, since this is more economical than other methods, reduces the silica content and removes suspended impurities. Effluent from an acid regenerated resinous exchanger is almost free from metals, but contains carbon dioxide, sulphuric and hydrochloric acids. The carbon dioxide may be eliminated by aeration and the other acids by

neutralization with alkali or the use of a resinous anion exchanger. Numerical details are given of the quantities of regenerating chemicals required after definite quantities of water with known mineral content have been treated and of the costs of operation, regeneration and replacement of the used resins. In the opinion of the author, the amount of effort and money being expended now in the production of new ion exchangers is not justified by the probable future demand for these substances.

T. J. W.

Australian Bentonite. C. LYNCH (*Chim. et Industr.*, 1949, **61**, 189). Deposits of bentonite and fuller's earth are found in Australia in Queensland, New South Wales, Victoria and Western Australia. The largest, in Queensland, is estimated at 28,000 tons (with 122,000 tons of bentonite clay). Bentonite is of two kinds, expanding and non-expanding. Expanding bentonites are used mainly as binders in the sand moulds of foundries, in which capacity they allow coarser sand to be used and so permit the escape of steam and gas from the cooling metal. Bentonite is used as a seal to strengthen embankments and excavations and prevent ingress of water. Expanding bentonite is also used for de-inking newsprint, clarifying wine, as a binder for pottery, as a dispersing and adhering vehicle for insecticidal dusts, for clarifying and removing flavour from water, for dehydrating wood pulp, and for emulsifying asphalt. Non-swelling bentonite is used for clarifying and purifying animal, vegetable and mineral oils. The viscosity of the treated oil tends to increase. Fuller's earth is used in petroleum refineries for the removal of colour and impurities, sludge, carbon, and acidity. It lowers the viscosity of the oil. It is also used to remove unpleasant odours and flavours from animal and vegetable oils and fats.

A. A. D. C.

Fungicidal Treatment for Cork Gaskets.

S. BERK (*Industr. Engng. Chem.*, 1949, **41**, 627-633). Since composition cork composed of granules bonded with adhesives containing protein or resin with glycerine is liable to attack by deleterious moulds if kept in warm, damp situations, the author has investigated the fungicidal effect of various preparations in suppressing mould growth. In addition, the effects of the treatment upon deterioration of the cork, the corrosion of metals in contact with the treated cork, the toxicity to those handling the material, and the effect of leaching with water upon the fungicidal properties were studied. The moulds used in the tests were *Aspergillus niger*, *Chaetomium globosum* and species of *Penicillium* and *Trichoderma*, and the fungicides were *p*-nitrophenol, 3, 5-dinitro-o-

cresol in various concentrations and solvents, and malachite green oxalate. No one of the fungicides tested fulfilled all the requirements necessary, but the most satisfactory was treatment of the corks with 2 per cent. aqueous *p*-nitrophenol solution, and this was recommended for use, although it caused slight corrosion when in contact with aluminium or steel. In general, the resin-bonded cork formed with thermosetting phenolformaldehyde resin offered greater resistance to the growth of moulds than did the protein-bonded cork. As a result of these tests it is recommended for future work that resin binders should replace those containing protein, the fungicide should be incorporated in the binding material and the fungicide should be non-toxic to human beings.

T. J. W.

Beer Cartons: Tests for Evaluation.

B. H. NISSEN (*Proc. Amer. Soc. Brewing Chem.*, 1948, 95-101). The increased use of corrugated cardboard cartons has caused manufacturers and users to devise methods of testing cardboard, its components, and the finished cartons. The most important single factor in strength is the fabrication. The corrugations must be of uniform height and form, and placed in a constant relationship to each other. Gluing must be uniform and the bonding strength at least equal to that of the paper liners. Moisture is important in that low moisture content, in general, improves the bursting and tensile strength characteristics, while high moisture improves "stretch," folding ability and tear resistance. Thus control of humidity is desirable for consistent plant working qualities and essential for testing purposes. Test standards for the industry as a whole have not been set up, but many users and makers use the tests summarized below. Laboratory tests for corrugated board and its components, cut from the cartons as received include: (1) Gauging for thickness and determination of the weight per 1,000 sq. ft. (2) Moisture content by drying at 105° C. for 24 hours. (3) Physical inspection of flute form and regularity, and of bonding and uniformity of gluing. (4) The Mullen Test for bursting strength. (5) Puncture Test. (6) Stiffness Test. (7) Flat Crush Test. Tests (4)-(7) are carried out with machines designed for the purpose. Five tests are described for finished cartons: (1) Measurements. (2) Physical inspection. (3) The Drop Test, carried out using a device which drops loaded cartons in a uniform manner from a predetermined height. Drops can be made on corners, edges or flats, so that the test simulates damage incurred in handling and shipping. (4) The Conbur Impact Test, simulating the ability of the carton to protect its contents. (5) The Compression Test which utilizes a machine designed for the purpose and

tests the ability of the carton to withstand pressure, such as that which might result from stacking. Comparative figures on board and board components are given to illustrate some of these tests. To establish specifications for beer cartons, some standardization of methods would be necessary.

C. R.

V.—BIOCHEMISTRY

Comparison of Maize Starches at Various Stages of Kernel Maturity. M. J. WOLF, M. M. MACMASTERS, J. E. HUBBARD and C. E. RIST (*Cereal Chem.*, 1948, 25, 312-325). This work was carried out on Iowa Hybrid 939 dent and Iowax 1 hybrid waxy dent maizes, and on Golden Cross Bantam sweet variety maize, grown in 1945 and 1946. Its object was to determine the approximate rate of starch formation in maize endosperm, and some physical and chemical properties of the starch granules at different stages from 12 to 13 days after pollination until maturity of the kernels. The most rapid increase in starch content of the grain of all three varieties took place in the period from 12 to 20 days after pollination. The average diameter of the starch granules in the period from 13 days after pollination to maturity increased from 2.7 to 9.9 μ in the non-waxy, from 3.0 to 9.1 μ in the waxy, and from 1.8 to 3.6 μ in the sweet maize. The water-binding capacity of the starches at 12 to 13 days after pollination was about 0.9 gm. per gm. of starch, and fell rapidly to about 0.3 gm. per gm. at 20 to 36 days. Over the period studied, the iodine-absorptive capacity of the non-waxy maize increased from 10 to 14 mgrm. per gm. to 54 mgrm. per gm. Fractionation of the starch from sweet maize 12 days after pollination confirmed the fact that it had a low amylose content. There was sufficient variation in granule size at the different stages of maturity to show the dependence of light transmission on granule size. The light transmission-temperature curves for the waxy maize starches were of simple sigmoid form, showing an increase in transmission with increase in maturity of the grain over the entire temperature range. The non-waxy maize starches showed correlation between light transmission and granule size only in the pregelatinization range of temperature, and sigmoid curves were given only by starches from immature kernels. At maturity the curve showed two inflections, with a region of relatively low light transmission between 75° and 90° C. (167° and 194° F.).

A. A. D. C.

Electrolytes as Constituents of Starches, and their Function in the Hydrolysis of Starches by the Amylases of Barley and Malt. H. LE CORVAISIER (*Bull. Soc. Chim. biol.*, 1948, 30, 202-213). The first part of this

paper is devoted to a survey of the work regarding the amylose-amylopectin constitution of starch, and the nature of the enzymes in barley and malt. The author then describes his own experiments, in which he has followed the de-mineralization of a series of different starches (cereal and root) and soluble starches in the course of their hydrolysis by barley diastase and malt diastase at different temperatures. Samples were taken at intervals and the degree of hydrolysis ascertained by determining the maltose formed. At the same time the amounts of total mineral matter and ionized phosphate liberated by the hydrolysis were found by analysis of the unchanged starch after electro-dialysis. With malt diastase there was a rapid liberation of electrolytes in the early stages of hydrolysis, but the rate of liberation of phosphate diminished as hydrolysis proceeded, so that there was a greater proportion of phosphate in the residual dextrin than there was in the original starch. The proportion of phosphate to total ash was also changed; the ratio phosphate/ash in the residual dextrin was higher than in the starch, showing that there had been a greater loss of cations than of anions (which is what has been found to happen during the preparation of soluble starch). The same results were obtained with both large and small granules of the same starch, except that, since the small granules contained more mineral matter than the large ones, there was a greater liberation of electrolytes at the outset of hydrolysis. The only difference between the various starches was that liberation of electrolytes from root starches was slightly slower throughout than it was from cereal starches, which suggests that the mineral matter in root starches is more closely bound up with the carbohydrate than it is in cereal starches. With barley diastase there was very little liberation of electrolytes. From these results the author deduces that barley diastase attacks only the outer, less mineralized portions of the starch granules, whilst the attack by malt amylase is more penetrative and therefore liberates more electrolytes. With the first there is almost no liquefaction, nor need for it, since the products of hydrolysis contain little electrolyte and have therefore a low viscosity. With the second there is much liquefaction, necessitated by the high viscosity of the products of hydrolysis with their comparatively high content of electrolytes. The author therefore considers that there is no need for the idea that starch contains two distinct fractions, amylose and amylopectin, but that these substances are merely depolymerization products of starch. There are, certainly, differences in the degree to which the carbohydrate in the granule is condensed. Condensation increases from the outside inwards, and is associated with increasing content of electrolytes, which largely determine

the physical properties of the starch. Liberation of these electrolytes, especially the cationic portion, by enzyme action is the result of liquefaction; saccharification liberates very little, and that mainly anionic. It is therefore questionable whether two distinct amylases, one dextrinogenic and the other saccharogenic, exist. The author prefers to consider that liquefaction is produced by an amylophosphatase and that hydrolysis proper is the work of a single amylase. These views do not conflict with the hypothesis that starch contains straight-chain α -glucosido-glucose with 1:4 linkages and branched chain α -glucosido-glucose with 1:6 linkages. The latter represents the more condensed portion of the starch granule, amenable to attack only by the diastase of malt.

A. A. D. C.

Limit-Dextrinase Activity of Barley Malts. E. KNEEN and J. M. SPOERL (*Proc. Amer. Soc. Brewing Chem.*, 1948, 20-27). A procedure is described for the evaluation of limit-dextrinase activity of malts and the importance of the enzyme (which converts unfermentable limit-dextrins to fermentable sugar) to the distiller is discussed. The substrate for the evaluation is standard limit dextrin prepared by hydrolyzing gelatinized potato starch with a mixture of bacterial α -amylase and purified barley β -amylase. Sugars are removed from the hydrolyzate by yeast fermentation, the liquid evaporated, clarified with active carbon and the limit dextrins precipitated by 75 per cent. propanol. The gummy precipitate is repeatedly worked with methanol, dried and powdered. For enzyme evaluation, 10 ml. of malt infusion is allowed to act on 20 ml. 3 per cent. limit dextrin for 2 hours at 55° C. and p_H 4.95. For higher limit-dextrinase activities, 5 ml. malt infusion plus 5 ml. 0.5 per cent. sodium chloride is used. The infusion is prepared by extracting 10 gm. finely ground malt for one hour at 30° C. with 100 ml. 0.5 per cent. saline. Limit-dextrinase activity is expressed as apparent maltose, determined by alkaline ferricyanide.

The activity of barleys on malting remains low until after the first day of germination, after which it increases rapidly until the 4th day. A decrease in the rate of development of the enzyme takes place after the 5th and 6th days. The activity is unimpaired by kilning temperatures up to 140° F., loss occurring progressively at higher temperatures. Activity also varies considerably with barley variety. High alcohol yields from maize are associated with high limit-dextrinase activity of the malt used for conversion, but no relationship exists between alcohol yield and diastatic power or α -amylase activity. There is as yet no application of this particular activity to the brewer. The activity

of limit-dextrinase may be due to its power of attacking 1-6-glucosidic linkages of limit dextrin, which are not attacked by α - and β -amylases.

C. R.

Enzymatic Activity Relative to End Fermentation of Malt-Converted Maize Mash. P. R. WITT and R. L. OHLE (*Proc. Amer. Soc. Brewing Chem.*, 1948, 28-35). High amylase activity is not necessarily indicative of maximum alcohol yields from malt-saccharified mashes and data are presented confirming the presence of a limit-dextrinase in malts (see preceding Abstract). The data were obtained by use of a technique of "secondary conversion" of mashes, in which premalted, cooked maize mashes were converted with 4 per cent. of malt slurry at 62° C. for 30 mins., the temperature thereafter being raised 1° C. each min. to 82° C. and held at this level for 15 mins. The mashes were cooled, adjusted to p_H 4.7 and fermented at 30° C. for 48 hours. After removing alcohol by distillation, the dealcoholized beer was adjusted to p_H 5.1, diluted to the original volume, reconverted with 4 per cent. of malt slurry at 62° C. for 5 mins. and again fermented.

Dealcoholized beers remashed with low temperature kilned malts gave significant quantities of additional alcohol, in contrast to those remashed with malts which had been finally dried at 170° F. for 12 hours or 190° F. for 5 hours. Remashing with malts inactivated with respect to α -amylase by treatment at p_H 3.6 (this *Journ.*, 1927, 178) did not give reduced alcohol yields. Double mashing with β -amylase-inactivated malts gave alcohol yields 45 per cent. below normal and the limited alcohol derived from the second mash appeared to arise from α -amylase conversion. Reconstitution of β -amylase inactivated malts with purified β -amylase resulted only in a partially regained alcohol yield. It appears that an enzyme (or enzymes), other than α - and β -amylases, is active in alcohol production. The enzyme is heat sensitive, stable to p_H 3.6, and maintains its activity throughout the fermentation period.

C. R.

Soluble Carbohydrates in Grass. T. J. DE MAN and J. G. DE HEUS (*Rec. Trav. chim. Pays-Bas*, 1949, 68, 43-50). Direct determination of soluble carbohydrates was undertaken for the evaluation of grass as fodder. The scheme of analysis was tested on the first growth of *Lolium perenne*, cut in June and dried in a current of hot air. Two fractions were prepared for analysis: (1) 2 gm. air-dried material was extracted overnight at room temperature with 80 per cent. alcohol, followed by a second extraction for 30 mins., and the combined extracts evaporated to dryness in vacuo. The residue was taken up in water,

neutralized to litmus, clarified with lead acetate, the filtrate freed from lead with secondary sodium phosphate and neutralized. (2) The residue from (1) was extracted with boiling water, the extract clarified with lead acetate and treated subsequently as in (1).

Total reducing power and fructose were determined on each fraction before and after hydrolysis. The method of drying and of alcohol extraction minimized the hydrolysis of fructosan to fructose. Differentiation of fructose derived from sucrose and from fructosan was possible, since fructosan was insoluble in 80 per cent. alcohol, which almost quantitatively

extracted reducing sugars and sucrose. An estimate of aldoses was obtained from the difference between free fructose and the total reducing sugars after hydrolysis. Sources of glucose, other than sucrose, were present only in small amounts. The contents of fructose, sucrose and fructosan were respectively 1.2, 5.9 and 3.3 per cent. A study of the variation of these carbohydrates with age of the grass revealed that fructosan passed through a peak. Analysis of samples of grass harvested at different times of day showed that only sucrose was subject to diurnal variation, the content increasing with exposure to light. C. R.

REVIEWS

Micro-organisms and Fermentation. By the late ALFRED JÖRGENSEN. Rewritten by ALBERT HANSEN. Seventh English Edition. London: Charles Griffin & Co., Ltd., 1948. Price 60s.

The original edition of Jörgensen's famous book, and the later translations into English are too well known in this country for any retrospective praise or detailed description to be necessary. Many editions have appeared since it was first published in 1886, and have enjoyed the greatest popularity, not only amongst brewers, but also those engaged in other fermentation industries. The present edition—the fifteenth in all, and the seventh in English—has been largely rewritten by Albert Hansen; indeed, as stated in the preface, the chief reason why it is still published under Alfred Jörgensen's name is that it still largely follows the general plan on which the original and subsequent editions were based.

On glancing through the new book, one is at once impressed with the extremely clear and readable style. The translator, Axel Anderson, is to be congratulated on his English—his plain English—for there is a welcome absence of that scientific jargon in which so many present-day scientific writers seem to glory, and the whole text exhales a pleasant enthusiasm for the subject.

In view of this, and of the popularity enjoyed by Jörgensen's previous editions, it is a matter of great regret to the reviewer that unqualified praise cannot be given to the present volume. For it must be recorded that, even on casually dipping into the book, one encounters an excessive number of misprints, mistakes, misquotations and inaccuracies, whilst even more are to be found on reading with closer attention. Some of these errors are, admittedly, somewhat

self-evident, and therefore need not mislead the experienced reader; others, however, are obvious only to those who already know the subject. But one cannot help feeling that a student, detecting mistakes in a part of the book where the subject is already familiar, will be bound to suspect the accuracy of unfamiliar sections when information is being sought.

Of the less important type of mistake, the following are a few examples: R. H. Mees is referred to as "Mess" (three times). *Leuconostoc dextranicum* Hucker et Pederson is referred to as "*L. dextranicum* Hycker et Pederson," whilst *A. anaerobium* has become "*A. anaerobicum*." *L. pastorianus* is given as "*L. pasteurianus*" (six times), this being stated to be "according to the nomenclature used in Bergey's Manual of 1939" which, of course, is not the case. On page 487 a warning is rightly given not to confuse "*Termobacterium*" with *Thermobacterium* (meaning heat-loving). On page 422, however, *Termobacterium bulgaricum* is the name given to this well-known thermophile, whilst the error is perpetrated in reverse in regard to *Acetobacter rancens* and *Termobacterium aceti*, which are stated to be "included under 'thermobacteria' (see page 486)." To make this still more puzzling, page 486 contains no mention of thermobacteria whatsoever. Rather more serious to the student in search of accurate information are categorical statements of the following kind: "*Pseudomonas* species are monotrichic"; "The disinfectant effect of soap is due to its content of alkali"; "*Phycomycetes* (the algae, lower fungi)"; "After some opposition, Hansen's system was soon accepted and adopted in all brewing countries."

One other feature of the book demands mention. The author has a habit of commencing a subject with a feasible but inaccurate

theme, statement or analogy, and then proceeding, by unwittingly contradicting it in the ensuing paragraphs, to engender doubts as to its validity. For instance, the section entitled "Spore Formation" opens with this sentence: "Just as the cell division of bacteria may be looked upon as a parallel to the asexual propagation of yeasts and moulds, the spore formation of bacteria may presumably be said to correspond to the sexual propagation of those organisms." Although both the premise and the conclusion in the above analogy could scarcely be more inaccurate, the student may find them plausible. Unless critically minded, and well advanced in his subject, he may fail to realize that there is no more resemblance between the clouds of (asexual) conidia produced by *Penicillium*, and the division of a bacterial rod into two, than there is between the complicated copulation of yeasts and the simple, solitary, asexual spore of bacteria.

The prevalence of such blemishes as the above in an otherwise well-written work, makes it difficult to arrive at a fair and accurate assessment of its general value. One feels an increasing nervousness, even when reading otherwise excellent passages, lest one should suddenly stumble on some error that will destroy confidence in the rest of the text. Perhaps the fairest judgment would be to say that, to the brewer, or to the corresponding technologist in other fermentation industries, anxious to acquire a broad grasp of the fundamental scientific basis of his work, the book may be confidently recommended as supplying all, and perhaps more than he needs. Apart from the blemishes mentioned, he will find it stimulating, extremely readable and easy to understand, even though it is chiefly Continental practice that is described.

On the other hand, the scientific student who wishes to adopt Industrial Microbiology as his subject, may find the book of chief value for its authentic presentation of the historical background and general atmosphere of traditional Continental (particularly Danish) "Fermentology." For accurate reference purposes

he should perhaps augment his study of the book by reading the work of the appropriate specialists on the various sub-divisions of this very large subject.
J. L. SHIMWELL.

Absorption Spectrophotometry. By G. F. LOTHIAN, M.A., F.Inst.P. London: Hilger & Watts, Ltd., 1949. Price 26s.

This work on absorption spectrophotometry was apparently begun as a third edition of Troyman & Allsopp's well-known volume, "*The Practice of Absorption Spectrophotometry with Hilger's Instruments.*" Virtually, however, it is a new work. Since the last edition of the book in 1934 great advances have been made in this subject with corresponding ramifications into many new fields. In view of these fresh advances in knowledge of this subject, the new author and publishers have wisely taken the course of producing a new work. It is true that portions of the two former editions have been incorporated, but the insertions have been skilfully carried out.

The first five chapters [which compose Part I] explain the principles of spectrophotometry, and the reviewer is lost in admiration at the author's presentation of a difficult subject. The treatment is logical and even the least mathematically-minded will be able to face the various formulae with only a slight sinking feeling. Part II is concerned with the applications of spectrophotometry and could with advantage in a new edition be extended, especially Chapter VII on Biological and Biochemical Applications. Part III is devoted to the practical side of the subject and here no fault can be found in the treatment.

There is an authors' index and the bibliography is adequate. The book is printed on art paper and illustrations and graphs are well and boldly reproduced. The author and publishers are to be congratulated on an excellent production which will no doubt rank as a standard work on the subject. The price of the book in these days of disguised and undisguised inflation is not unreasonable.

E. BARTON-WRIGHT.

JOURNAL OF THE INSTITUTE OF BREWING

MONTHLY REVIEW

EUROPEAN BREWERY CONVENTION LUCERNE, 1949

UNDER the Presidency of M. Philippe Kreiss (France) and the extremely able secretaryship of Dr. F. Mendlik (Holland) success was only to be expected for the Congress held at Lucerne from 29th May to 2nd June, 1949, but the local Swiss Organizing Committee under the Presidency of Dr. Kurt Schöllhorn ensured that absolutely nothing was left to chance. Organization was perfect and the occasion was an unqualified success. Well over 200 delegates attended from 15 or more countries and with their ladies there were altogether some 325 persons, mostly housed in three of the large hotels facing the lake.

The business and scientific meetings were held in the magnificent and admirably equipped *Kunst und Kongresshaus*, and the proceedings opened on Sunday evening, when Dr. Schöllhorn welcomed the delegates on behalf of the *Schweizerischer Bierbrauerverein*. Subsequently there was an opportunity for greeting old friends and making new acquaintances. The company was interested to see two Swiss films publicizing beer; while these films may be suitable for the particular conditions of Switzerland, the impression was that they would not achieve their object in this country.

There were four scientific and technical sessions and one business session. For the technical sessions there were two main subjects for discussion, besides the reports of the Barley Committee. Most of the papers having been pre-circulated, it was possible in most cases for the speakers to give a brief resumé of their contributions, discussion following.

At the first two sessions, the subject was *Proteins in Brewing*, and the opening contribution was a general report by Professor H. Lundin (Stockholm), who provided a most valuable comprehensive survey of the whole

field. The paper cannot be adequately summarized, but it covered such topics as enzymes and proteins of barley, malt and yeast, and transamination of amino-acids. These last reactions are of immense importance in brewing, since the nature of the amino-acids and their reactions determines the character of the fusel oils formed. Apart from flavour, the effect on surface film and hence on head retention is a very practical result of these reactions. Reference was made to current investigations of nitrogen absorption by top and bottom yeasts and to the use of the radio-active isotope technique in the study of amino-acid assimilation. N. Kent and M. Macheboeuf (Paris), in *Recherches sur les Protéines de la graine d'orge*, discussed the isolation of certain barley fractions subsequently identified by paper micro-chromatography. This was followed by E. C. Barton-Wright's (London) *Nitrogenous Constituents of Wort and their fate during Fermentation by top and bottom Yeasts*, in which he discussed the chief nitrogenous components of wort and described his work on the behaviour of some 16 of the wort amino-acids during fermentation, studied by means of microbiological assay. B. Trolle (Copenhagen), discussing *The action of Pepsin in Beer*, showed the pronounced effect of the enzyme (previously freed of papain and other proteolytic enzymes) on chill haze and oxidation haze proteins; this action occurs almost entirely during pasteurization. In the subsequent discussion it was stated that a reduction of 10-15 per cent. in head retention resulted from enzyme treatment, as described in the paper. Using Blom's method, it was always possible to detect even the smallest effect of added enzyme. The final paper of the first session by B. D. Hartong (Amersfoort) on *Properties and Composition of Chill Haze*, put forward a thesis, based on experiments with hop tannin, that the stability of beer rests on dehydration and re-hydration.

The second session was opened by J. H. St. Johnston (Burton) with his paper on *The Flocculation of Proteins in Wort and Beer*, which gave an account of his separation by fractional precipitation of proteins designated T, C and O, and described their characteristics (this *Journ.*, 1948, 305). It was contended that the results indicate that the mutual flocculation of colloids provides a working hypothesis for tackling many of the practical problems of brewing. In the ensuing discussion the point was made that perhaps the fractions T, C and O would be better characterized as "nitrogen complexes" than as proteins. E. Sandegren's (Stockholm) paper on *Investigations of Barley Protein in Brewing* provided a natural sequel to the same author's important contribution on the subject given at the Scheveningen Congress. The salt soluble proteins in barley had been fractionated and examined polarographically. Space does not permit of the adequate description of Sandegren's experiments and results, but his paper is of the expected high standard. *Les Protéines de la Levure*, by S. Brohult (Appelviken), described experiments in the extraction of brewer's yeast by three different methods. The extracts were analysed by the ultra-centrifuge and at least three different components were detected, with sedimentation constants of 9, 7 and 2.8. L. R. Bishop (London) followed with an account of his separation of the compounds of worts, intermediate between the proteins and the amino-acids. The technique was to employ increasing proportions of phosphotungstic acid (this *Journ.*, 1949, 147); as the concentration of the acid increases, the nitrogenous components precipitated become progressively simpler. *L'aération en Malterie et son influence sur la composition azotée des Malts*, by F. Tombeur and G. van Roey (Louvain) presented a communication of obvious practical importance, wherein it was contended that in pneumatic malting the quantity of air required for regulating temperature is far more important than that needed for respiration. By resolving the equation for the thermal equilibrium of the chemical reactions involved in germination it is possible to compare the different amounts of air during the whole germination period; aeration particularly promotes rootlet development, while temperature mainly influences respiration. The best malts are

obtained by germinating at low temperature in the presence of abundance of air. Most of the papers read at these two sessions were followed by free and interesting discussion, and it was not surprising, perhaps, that at the end of this section M. Kreiss moved from the Chair that a sub-committee of the Convention be appointed to consider the question of terminology. The deliberations of this sub-committee, consisting of L. R. Bishop, S. Brohult and M. Macheboeuf, will, it is hoped, clear the air regarding the names, letters and symbols being given to the various complexes, fractions and constituents described in the ever-increasing investigations in this field.

At the third session, devoted to the reports of the Barley Committee, H. van Veldhuizen (Rotterdam) opened with accounts of the research and investigations that are going on regarding the cultivation of malting barley in Holland, Belgium and France, and, more briefly, in the United States. He was followed by H. Thunaeus (Stockholm), who discussed the same questions in relation to Sweden and Central Europe. T. Bendixen (Oslo) followed for Norway, E. Vestergaard (Söllested) for Denmark, and L. R. Bishop (London) for Great Britain. P. Bergal (Maule), Director of "Secobrah" (*Société d'Encouragement de la Culture des Orbes de Brasserie et des Houblons en France*) pleaded that the following three points should receive absolute priority: (1) The perfecting of an international standard in order to fix, on a common basis, the criteria of quality of a brewing barley; (2) standardization of experimental methods for determining the inferiority or superiority of old or new varieties bred in each country; and (3) study of dormancy in relation to the important questions of drying and humidity. These papers were followed by lively discussion ranging over a very wide field, including the question of difference of taste resulting from the use of two-rowed and six-rowed barleys, the reasons for the use of six-rowed barleys for brewing in the United States, the banning of winter barley in Holland because of its harbouring of disease, the influence of climate on insect and fungus troubles, and many other topics.

The fourth session dealt with *The production of Sterile Beer by other means than Pasteurization*. H. Kringstad (Oslo) opened with a general report, reviewing the present

state of knowledge in the various methods of sterilization. After discussing the various effects and disadvantages of heat treatment, the author considered the possibilities: sterile filtration, sterilization by X-rays, ultra-violet rays, electronics and supersonics, and the use of antiseptics. Less discussion followed this paper than the subject merited. On the more restricted subject of the next contribution, *The Use of Hydrogen Peroxide as a Beer Preservative*, by H. R. Jacobsen and J. Nestas (Oslo), however, there was less shyness in debate, while the following paper by A. Juillerat (Zurich), *Essai sur la Stérilisation des Bouteilles à Bière au moyen de Rayons Ultra-violet*, led to a most exhilarating skirmish. The session concluded with an account by G. Osgood (Ashton-under-Lyne) of *Sterile Filling*.

The remaining session of the Convention was devoted to formal business. The Secretary's report started with an historical outline and proceeded to give an account of the business conducted at the various Council meetings since the Scheveningen Congress in 1947. Of particular interest were the references to the Analysis Committee which has the task of considering the possibility of standardizing methods of analysis in malting and brewing, and those to the main subjects to be discussed at the next meeting of the Convention in 1951, to which further reference will be made below. An interesting discussion on the nature of the meetings took place. While the opinion was voiced that, in view of the technically scientific nature of some of the contributions, future meetings should be split into two sections, one for the scientists and the other for the practical brewers, the impression gained was that most delegates viewed this as a retrograde suggestion: that the purposes of the Convention meetings would best be served by bringing the different aspects closer together. The feeling of the meeting was probably more accurately gauged by the speaker who considered it was desirable that the "laboratory man" should present his scientific contribution to the meeting, but should make it acceptable to the practical man by explaining how his laboratory findings were translatable into brewing practice.

Those who attend congresses and conventions know that it is no idle cliché that a valuable part of such occasions consists of

the contacts and the re-contacts made. This Convention proved the truth of this; at Lucerne the opportunities for numerous unofficial talks and discussions were unsurpassed, and they were aided by the hospitality of the hosts, the Swiss Brewers' Society. On the afternoon of the first day the Lucerne Brewery *zum Eichhof* was visited, a magnificent snack buffet at the *Kursaal-Casino* following. On the afternoon of the third day the opportunity was given, and widely accepted, of visiting one of a number of breweries in different parts of the country. Hospitality was as open and lavish as it possibly could be. Apart from organized brewery outings, many delegates took the opportunity of unofficial visits. The kindness of certain brewery directors and brewers who gave up some hours of their private time to their foreign visitors will not readily be forgotten. The purely social functions were organized as thoroughly as the business proceedings. On the afternoon of the last official day of the Congress, the Swiss Brewers' Society provided an excursion by special boat on the Lake of Lucerne to *Tellsplatte*, and on the same evening was held the banquet in the *Kunsthaus*, delegates again being the guests of the Swiss Brewers' Society. Gastronomically and socially this gathering was a climax. There may be recalled with particular pleasure the clear and happy remarks of the Mayor of Lucerne, the speech of Mrs. Hartong on behalf of the ladies, for whom special social activities had been arranged during the Congress period, and the contributions of the two British speakers, Mr. W. J. Watkins and Mr. B. M. Brown.

To sum up: The European Brewing Convention at Lucerne, 1949, was a success from every point of view. This was in large measure due to the standard of the contributions to the meetings, to the skill and organizing ability of the officers, particularly M. Kreiss and Dr. Mendlik, and the perfect work of the Organizing Committee of the Swiss Brewers' Society. It is proposed to hold the next meeting of the Convention in Britain; it will be difficult to emulate the Swiss in their organizing ability and hospitality, but every endeavour must be made to see that the standard set at Scheveningen and maintained at Lucerne is upheld in Britain in 1951.

The full proceedings of the Convention,

with the text of the papers (including some read by title only) and the discussions are expected to appear in the autumn of this year, and abstracts of the papers will appear in later issues of this *Journal*.

It remains to acknowledge the help derived from the presence at the Congress of Miss Pamela Bell, of the Institute Staff, who was always available to assist members of the British contingent.

The Next Convention Meeting.—The two major subjects selected for discussion at the next Congress are in the one case of a scientific character, and in the other of a more technical nature, from the point of view of brewing practice. They are:

- (a) The flocculation of yeast during the main fermentation.
- (b) Aspects of the economic functioning of the brewery, subdivided as follows:
 - (1) Losses of material (i) in brewing, (ii) in racking and bottling.
 - (2) Mechanization and new technique in the brewery and bottling plant.

The Secretary of the Convention, Dr. F. Mendlik (P.O. Box 455, Rotterdam) is already requesting that those people making a study, or intending to make a study of these subjects, should get in touch with him, so that he can ensure that the results of such work can be made public and discussed at the 1951 Congress.

H. J. BUNKER.

* * * *

SCIENCE *versus* STARVATION

It has been obvious for some time that the primary need in the treatment of a world suffering from post-war shock is the production of more food. Lord Boyd-Orr has given warning of the risk of world starvation in the future, and the United States has not been without her prophets of calamity. It is heartening, therefore, to find a distinguished American scientist, Dr. C. S. Miner, championing the cause of science *versus* starvation in an address given to the American Chemical Society and the Society of Chemical Industry in New York early this year. The occasion was the presentation to Dr. Miner of the Perkin Medal in recognition of his achievements in applied chemistry, and the address has been published in the May, 1949, number

of *Industrial and Engineering Chemistry* (pages 963-967).

The first great aid of science to food production, says Dr. Miner, is in the selection of improved strains of food plants, and he cites the introduction of hybrid maize as an example. The improvement in malting barley over the last fifty years would serve as another. In less direct ways science is busy increasing food supplies by discovering more potent substances with which to safeguard growing and stored foods from attack by fungi, insects, rats and other pests, to remove the crippling competition of weeds, and to prevent or postpone the onset of rancidity in fats. By studying the problem of how to control heating in stored foodstuffs it will eventually prevent a great deal of loss; the particular difficulties attached to the storage of peanuts will no doubt be of special concern to us some day. 'Cold storage has long been a powerful weapon against the agents of food spoilage, and now the process of quick freezing is becoming important in preserving the nutritive values of the more perishable kinds of fruit and vegetables.

Dr. Miner goes on to discuss how we can use food to the greatest advantage. We will do this, he says, if we "reduce our intake of animal foods and increase our intake of cereals," because the conversion of animal feeding-stuffs to eggs, milk, beef or mutton is a wasteful process. As an American, Dr. Miner's knowledge on this point is probably largely theoretical, and we in this country, who have been experiencing such a regime for what seems an inordinately long time, can assure him that it has little to commend it beyond its soulless efficiency. One may ask, moreover, whether animal husbandry has not a place in most farming systems, which would be badly put out of balance if there were too marked a move towards world vegetarianism. We feel more in tune with Dr. Miner's views when he advocates an increased use of wood-pulp sugar and yeast for animal feeding, and calls attention to the discovery that simple ammonium salts and urea can satisfactorily replace up to one-third of the protein required by ruminants. Further, by selecting suitable strains of micro-organisms to be ingested with this non-protein nitrogen, it may be possible to use a still greater proportion, or even to include it in the diet of domestic animals other than ruminants.

Micro-organisms are to the fore in other ways. The value of yeast as a manufacturer of vitamins from simple substances is well known, and it has recently been disclosed that the "animal protein factor" (a substance needed in the diet of animals fed largely on plant protein) can be made on a large scale by means of a bacterial fermentation.

One final point from Dr. Miner's stimulating address has its sporting as well as its utilitarian aspect. The fish population of lakes and ponds can, it appears, be vastly increased by adding to the water fertilizers of the type used for plants. No details are given, and secretaries of angling clubs would, perhaps, be wise to seek them before attempting to act on this information.

* * * *

WYE COLLEGE DEPARTMENT OF HOP RESEARCH: REPORT, 1948

THIS report covers the period from March, 1947, to March, 1948. It is the first to be issued by the newly reorganized Hop Research Department at Wye College, and the intention is to make it an annual production. The first part of the report is of a general nature and deals with such matters as the increases in staff and accommodation consequent on the reorganization, the lay-out of the hop gardens and the planning of the work, and the behaviour of the hops during 1947. Brief descriptions are given of manurial and cultivation trials and work on new varieties. The question of whether the hop suffers from having its bine cut before withering, which is raised by the introduction of mechanical picking, is being examined anew, and some experiments on hop storage are summarized, from which it appears that rate of loss of α -resin in stored hops is to some extent a varietal character. This part of the report ends with a reference to the kind of advice which is sought from the department by hop growers, and to the purposes for which hop analyses have been made.

The rest of the report consists of articles on particular aspects of hop research. The first of these is a review of the literature on the nutrition of hops, and describes pot culture tests, trials on the effects of deficiency of various nutrients, the importance of soil reaction (which must not be allowed to fall below p_{H} 6.5), the manurial needs of the hop plant, and the relation between nutrition

and resistance to disease. A particular aspect of this last point is taken up in greater detail in an account which follows, of investigations made at Wye College during 1944 to 1947 on the influence of soil and nutrition on the incidence of *Verticillium* wilt. Analyses of leaves from apparently healthy Fuggle hops in healthy and infected gardens have shown significant differences in the levels of calcium, silicon and nitrogen, calcium and silicon being higher in infected gardens and nitrogen lower. Significant differences were also found in "moisture absorption" figures, which are considered to show that there is a difference in the physical properties of the leaf tissues. Alternative explanations for these differences are offered. Either the apparently healthy hops in infected gardens were, in fact, infected and the differences found were due to the presence of the fungus, or the hops in the healthy gardens were nutritionally different from the others and this difference made them less susceptible to attack. The results of artificial infection of some hops with progressive wilt gave no support to the first of these alternatives. Calcium was the element which differed most consistently in all comparisons, but there were no significant differences between the soils in healthy and infected gardens, so that the level of leaf calcium does not appear to be affected by the level of soil calcium. It may be that the calcium levels are characteristic of the hops examined, and that there are high and low calcium strains of Fuggles. It is suggested in the report that experiments might be done to see if this is the case, and if so, whether the strains with low levels of leaf calcium are less susceptible to *Verticillium* wilt. It might also be possible to alter these calcium levels by applying sodium salts to the soil, since sodium is known to depress the intake of calcium by plants.

The third and last article is a discussion of the problems that beset the hop breeder in his attempts to produce hop plants of better quality by hybridization. There are three stages in this work. The first, the production of as many hybrids as possible from which to choose plants of promise, has already been accomplished. The second stage is the recombination of variations to build up specified improvements, and involves breeding control through the male as well as the female line, followed by the lengthy testing of each set of progeny. Work on this stage is only just

beginning, and is complicated by the constitution of the hop plant, which—to quote the report—“seems peculiarly adapted for putting difficulties in the path of the research worker.” The third stage, which has not yet been reached, is the development of methods of inducing variations artificially. The report concludes with a summary of Prof. Salmon’s note on two new hops: “Early Choice” and “Concord Hop,” which had already appeared in abstract form in this *Journal* (1948, 109).

* * * *

PROGRESS REPORT: MIDWEST BARLEY IMPROVEMENT ASSOCIATION, U.S.A.

This report was presented at a Board of Directors’ Meeting held at Milwaukee in April, 1949. It describes briefly the 1948 Midwest Malting Barley Contest and Show and Barley Improvement Conference, all held at Minneapolis, and outlines the plans of the Association to offer prizes for malting barley at state fairs and other grain shows during 1949. By this means the Association hopes to draw the attention of growers to the financial value of crops of malting barley as distinct from feed barley. Reference is made to the experimental malting tests carried out on samples of barley varieties from each of the seven Midwest States, and to the comparative growths of Moore barley and a standard variety made by a number of farmers in Wisconsin. The types, number of growers and acreage of the 1948 crop of certified seed of malting barley for each of the states are tabulated, from which it is seen that the two varieties Kindred and Wisconsin 38 make up about 70 *per cent.* of the total. Figures are also given for the 1948 crops of Montcalm and Moore barleys. Seed crops of the latter were being grown in California and Arizona during the winter, and some of this seed may have reached the Midwest States in time for spring sowing.

An account is given of the “One Variety of Malting Barley” scheme, whereby all co-operating growers submit their seed barleys (other than certified seed) to the Association for variety analysis before sowing. Samples having less than 95 *per cent.* of the variety named, or with more than 1 *per cent.* of 2-row barley and/or Trebi, are “not approved.”

The report lists the state barley goals of the U.S. Department of Agriculture for 1949, the prospective barley sowings, the price supports, barley stocks on 1st April, 1949, in each of the Midwest States, and the financial aid which the Association is giving in the form of research fellowships and in other directions. The activities of the Association in sending representatives, speakers and barley exhibits to meetings and shows, and in writing and distributing information on malting barley, are summarized in three appendices.

* * * *

MANUFACTURE OF SCOTCH WHISKY

It is often of interest to glean information about an industry which in some of its technical details is related to one’s own, and for this reason an account of the manufacture of Scotch whisky will appeal to many of our readers. The following is based on a lecture by D. F. S. Hastie which was published in the *Journal of the Incorporated Brewers’ Guild* for May, 1949, and reference should be made to that publication for fuller details. Before dealing with the main subject the author gives a brief account of the history of distillation and of the origin of *aqua vitæ* (brandy) and *usequebaugh* (whisky), this being followed by a speculative, but probable, account of the discovery of distilled spirits. Although Scotch whisky has a world-wide reputation, there is evidence that the beverage originated in Ireland, for until about 200 years ago the spirit consumed in Scotland was brandy. The early stages in the manufacture of whisky closely resemble those of beer in that ground malt is mashed with hot water, but the wort is not boiled before being cooled and pitched with yeast so that fermentation takes place in the presence of diastase and at a temperature of about 73° F. Fermentation is completed in two days and various species of bacteria then become active and produce substances which contribute to the flavour of the finished product. The liquid now known as “wash” contains approximately 10 *per cent.* of alcohol and is transferred to a copper, fire-heated still fitted with a chain scraper and distilled until all the alcohol is collected in the receiver. The distillate called “low wines” is rectified by a second distillation in a similar but

smaller still, the first portion collected being the raw foreshots, the next potable spirit, and, finally, the objectionable feints; much skill is required on the part of the stillman to determine when to change the receivers for the three fractions. In addition to the pot still malt whisky produced by the above process there is another variety known as grain whisky which is made from a mixture of malt and raw grain and is distilled by continuous operation in a Coffey or patent still. This product has less character than the pot still whisky and is used for subsequent blending. As soon as the various distillates begin to run from the still condenser they are in charge of the Excise officers and are passed through a locked box with glass sides known as the "distillers' safe," which is so arranged that the stillman can collect samples and test the specific gravity, *etc.*, by manipulation from the outside. At this stage the whisky is a crude, fiery spirit and is transferred to oak casks in which it is allowed to mature for several years during which time it slowly loses its harsh character and becomes mild and mellow. Many procedures have been patented claiming to accelerate the maturation but none so far has proved successful. The whisky produced in any particular distillery has subtle characteristics which distinguish it from any other, and although attempts have been made in different parts of the world to manufacture Scotch whisky the results have not come up to expectation. The operation of blending entails a high degree of skill in the preparation of the completed beverage since any one brand may be a mixture of the spirit from several distilleries. Each blender works according to his own treasured formula and relies entirely on his educated senses of smell and taste, since chemical analysis is as yet useless in this connection. The choice of any particular brand by the customer is a question of taste and character since all genuine Scotch whiskies are equally good. The complaint not infrequently heard nowadays that whisky, like *Punch*, is not so good as it used to be is merely due to the lower alcoholic strength which is controlled indirectly by prices, duty and other factors.

* * * *

BREWING IN FINLAND

It is of interest, and not infrequently of profit also, to learn how one's particular

business is carried on in other parts of the world, and for this reason an article under the above title by Poul Ruus appearing in the *American Brewer* for January, 1949 (Part 1), will appeal to British brewers. This account describes the procedures and products of a few of the principal breweries in Finland, together with some details of the industry in general. Although the total population of the country is under 4,000,000 it contains 50 breweries and 16 maltings, giving employment to 1,500 persons. The A. B. Sinebrychoff brewery is the largest in the country and produces 122,000 barrels of beer annually. The malt used is made from Finnish Binder barley and the mashing liquor is very soft (2.5-5° total hardness), so that no preliminary treatment is considered necessary. Hops are used sparingly at the rate of 5 oz. *per* barrel, whilst the wort is brewed at a gravity of about 45° and diluted in the fermentation cellar to 23°. Fermentation is carried out at temperatures between 43 and 49° F. in aluminium vessels each holding 122 barrels; the beer is stored in tanks of the same metal for 4 to 6 weeks, then bottled and sold without pasteurization. A beer known as *svagdricka*, having a gravity of 18° is produced in a novel manner, for after a fermentation period of only 24 hours it is bottled unfiltered, after which further fermentation proceeds, with the carbon dioxide produced escaping through a hole in the bottle neck covered with a rubber band. Evidently the Finnish breweries are less trammelled with regulations than are our own for at this one mead is made from Brazilian honey and flavoured with amyl acetate and lemon essence. In other respects we are better off than the Finns, for their product of a certain class is subject not only to the regular beer tax but also to alcohol and malt duties. A more modern brewery is the O/Y Mallasjuoma, to which is appended a pneumatic box malting where the malt is transferred from box to box by hand. In addition to beer this brewery manufactures malt extract, and molasses is used for the preparation of compressed yeast; but we are not informed if these are used in conjunction for domestic purposes as in the United States during the prohibition era. The O/Y Pynikki brewery, situated at Tammerfors, has four branches situated in various parts of the country, all of which are controlled by a central laboratory and supplied from a

maltings at Tammerfors. The production of beer by the five breweries amounts to 124,000 barrels, and nearly 2 million gallons of a mineral water flavoured with whortleberries are also provided. Finnish beers are of the Pilsener type and none is pasteurized, but they are relatively expensive since the high rate of wages paid to the employees reduces trading profits. Considerable modern developments in plant and machinery have been planned, but owing to delayed delivery of the necessary materials and equipment completion of these is not expected for several or many years.

* * * *

SOCIETY FOR GENERAL MICRO-BIOLOGY

The Society held a most successful symposium on "The Nature of the Bacterial Surface" at the Royal Institution on 20th and 21st April, 1949. After an introduction by Dr. N. W. Pirie (Rothamsted), Dr. W. T. J. Morgan (Lister Institute) spoke on the surface structure of *Shigella shigæ*, Professor M. Stacey (Birmingham University) on the nature of the surface of gram-positive bacteria, and Mr. Peter Mitchell (Cambridge) on the osmotic barrier in bacteria. Dr. T. F. Anderson of the University of Pennsylvania discussed the mechanism of adsorption of bacteriophages on host cells and Dr. A. A. Miles (National Institute for Medical Research) commented on the status of some arguments about the bacterial surface. Professor E. T. C. Spooner (London School of Hygiene and Tropical Medicine) dealt with the nature of bacterial surfaces, Dr. Harriett Taylor (Laboratoire de Genetique, Paris) with capsule formation in the pneumococcus, and Professor A. Pijper (Pretoria University) showed a film illustrating his views on flagella and motility. Dr. T. Y. Kingma Boltjes (Amsterdam), Dr. W. van Iterson (Delft), Dr. A. L. Houwink (Delft), Dr. D. McClean (Elstree), Sir Alexander Fleming (Paddington), and Dr. W. H. Hughes (Paddington) took part in the discussion. On the second day 18 original papers were given and there were four demonstrations.

At the Annual General Meeting of the Society the following were elected officers for the coming year: President: Prof. J. W. McLeod, F.R.S.; Hon. Treasurer: Mr. H. J. Bunker; Hon. Secretaries: Dr. J. G. Davies

(General) and Dr. W. E. van Heyningen (Meetings); Hon. Editors: Dr. B. C. J. G. Knight and Dr. A. A. Miles.

Full particulars of the work of the Society and of membership may be obtained from Dr. J. G. Davis, 35, Villiers Road, Southall, Middlesex.

* * * *

INDUSTRIAL CHEMISTRY

22nd INTERNATIONAL CONGRESS

It is announced that this Congress will be held at Barcelona during the period 23-30th October, 1949. It is organized by the *Société de Chimie Industrielle* in association with the Spanish Chemical Industries. The Congress is divided into 25 sections, some of which are of direct interest to readers of this *Journal*. Thus, Section 1 is devoted to analytical laboratories and their equipment and to methods of chemical and biological analysis, whilst the fermentation, agricultural, and food industries are dealt with in Sections 19, 20, and 21 respectively. Reservations may be made on application to the *Société de Chimie Industrielle*, Rue Saint-Dominique, Paris (VII°).

* * * *

RECENT ADVANCES IN THE FERMENTATION INDUSTRIES ST. ANDREWS SYMPOSIUM

THIS Symposium, organized by the Scottish Sections of the Royal Institute of Chemistry during the period 23rd-30th July, attracted an attendance of approximately 200, and was opened on Monday, 25th July, by Principal Sir James Irvine, F.R.S.

A full programme of lectures and social events was arranged, the lectures being divided under three headings. Section I (Monday and Tuesday) was under the Chairmanship of Mr. C. K. Mill, being devoted to *Edible and Potable Fermentation Products*. Of five papers under this heading, three referred directly to malting and brewing, being designed not so much to give a comprehensive description of these processes, but rather to bring out up to date information on certain selected topics. Thus, Dr. I. A. Preece, after briefly discussing the probable importance of changes in hemicellulosic materials during malting went on to discuss the α - and β -amylase relationships in the germinated

grain up to the time of kilning, this process stabilizing the proportions of the two enzymes within a comparatively narrow range. Mashing results in what is essentially an α -amylase conversion of the malt starch, with the products of reaction profoundly altered by the β -amylase, and the heat-sensitivity of the latter permits an important degree of control of the proportion of fermentable sugars produced. The total products of reaction include glucose and maltose, together with straight- and branched-chain dextrans of mean complexity equivalent to 7-8 glucose units; one or more trisaccharides may also be present.

Dr. L. R. Bishop referred to the particular interest of the globulin fractions of barley proteins during the brewing process, β -globulin being a protein found apparently only in barley and not in other cereals. It is the persistence of a β -globulin derivative in association with tannin which may make beer sensitive to chill and oxidation hazes. Reference was also made to the use of nitrous acid for precipitating beer protein and to St. Johnston's fractionation method. Fractionation of the nitrogenous compounds of wort and beer may also be made by the graded addition of phosphotungstic acid, and the results indicate the existence of compounds of varying complexity, containing approximately 1, 2, 4, 8, 15-16, and 30 *per cent.* of free amino nitrogen. Comparison of the results for wort and beer confirms that yeast utilizes only the simpler nitrogen compounds for its growth. Mr. E. Helm, comparing the behaviour of top and bottom yeasts, expressed the view that the former are the more exacting in their growth requirements, this being bound up with the higher temperatures at which they operate; further, the mixed nature of typical British yeasts enhances difficulties of study. The Continental barleys contain more nitrogen than the British, but the shorter growing time gives rather less permanently soluble nitrogen in the malts; ale malts, in general, are better modified than those used for lagers. Water treatment problems differ, and the lower p_H of British worts allows a greater use of hops; ale worts probably contain more nitrogen than those intended for lager. An experimental scheme was described for studying the influence of fermentation conditions; differences in pitching rate do not influence the final beer, but temperature change has an important effect.

Also in this Section, Mr. A. Olsen discussed the manufacture of bakers' yeast without alcohol production; some 1,000 tons of such yeast is produced weekly in this country. Probably the best raw material is molasses; after chemical or centrifugal purification this is sterilized and seeded with a pure yeast culture. Molasses and nutrients such as ammonia are added continuously, and the weight of yeast increases 5-7 times in 9-12 hours. Temperature and p_H are carefully controlled and growth is accelerated, and alcohol production prevented, by the admission of finely divided air. The yeast is separated centrifugally and is further de-watered by filter-pressing or by the use of continuous rotating vacuum filters. Dr. D. W. Kent-Jones dealt with panary fermentation, discussing fundamental work on dough properties and describing apparatus used for the routine evaluation of dough characteristics. The fermentation changes in dough were described with special reference to yeast growth, the decrease in amino acid content, flavour effects, and the influence of dough ripeness. Enzyme relationships are of considerable importance; in particular an excessive amount of α -amylase gives undue degradation of starch to dextrans, with production of a "soggy" loaf, whilst an inadequate supply of β -amylase—by giving insufficient maltose production—leads to a deficiency in gas production. Thus, for satisfactory results a careful balance of enzyme activity is essential.

Section II, *Industrial Fermentations*, was taken on Wednesday, the meetings being presided over by Prof. J. W. Cook, F.R.S. Production of industrial alcohol from molasses was discussed by Mr. E. R. Dawson, who noted that several main lines of advance had been developed in recent years. Thus, improvements have been made in the treatment of molasses prior to fermentation, whilst a number of continuous fermentation processes are now available. In one, the fermenting liquor passes successively through a number of fermenters in series, being completely fermented when it leaves the last; fresh mash is passed through till the yeast is exhausted. In a second method, two fermenters in series are used, the yeast separated from the fermented mash leaving the second re-entering the system with fresh mash in the first. Again, the process of "yeast re-use" suppresses the utilization of sugar for yeast

multiplication, and so gives enhanced alcohol yield; yeast is separated centrifugally at the end of fermentation and is used in the next fermentation.

In a comprehensive description of the butyl alcohol-acetone fermentation, Mr. E. Gill pointed out that economic success depends on the demand for butyl alcohol and butyl acetate in the lacquer trade. Development of the process has seen the change from starchy raw materials to molasses and the introduction of organisms with higher fermenting efficiency. Difficulties include problems such as losses due to bacteriophage, to which organisms producing low acetone ratios are particularly susceptible, and the disposal of waste liquors; however, concentrates of such liquors are rich sources of riboflavin.

Dr. G. G. Freeman dealt at some length with the 2:3-butylene glycol fermentation, the glycol—for which a number of industrial uses have been suggested—being obtainable by fermentations with three different bacteria, using starch or sugars (according to the organism chosen) as raw materials; different isomers or mixtures are obtained by the various methods, and a major difficulty is the ultimate separation of the glycol. Glycerol production by fermentation in presence of sulphite or alkali was also discussed, and means for the recovery of glycerol have now been developed to make the process an economic proposition. A paper of particular interest was that of Messrs. R. W. Jackson, R. H. Blom and H. M. Tsuchiya relating to the use of fungal amylase in industrial production of alcohol and alcohol products. Selected strains of certain moulds, especially of *Aspergillus niger*, when grown on a mixture of thin stillage and ground maize give a product which can be used to convert grain mashes prior to fermentation. The mould-amylase liquefies the starch, but alcohol production correlates better with one or more other enzymes, conveniently known as "maltase." Conditions for the economic production of the mould amylase have been worked out, and by its use alcohol yields are obtained at least equal to those with malt, and operational costs are substantially reduced.

Microbiological Advances formed the general title of Section III (Thursday and Friday evening), taken under the Chairmanship of Prof. W. H. Everett. Dr. J. H. Birkinshaw,

discussing fungal metabolism, showed how the oxidative, reductive, and synthetic capabilities of moulds have been studied. Of particular interest are changes involving citric, itaconic, and succinic acids, whilst the tetrionic acid series of products has obvious relationship with ascorbic acid and penicillic acid. A variety of aromatic compounds, some of which probably function in respiratory oxidation-reduction systems, are also encountered. Microbiological assay has in recent years proved a potent analytical weapon, and Dr. E. Barton Wright dealt with the subject in relation to determination of amino-acids. Strains of lactobacilli have proved valuable for the assay of vitamin B. components, lactic acid production within certain limits being proportional to the concentration of a particular vitamin, when a suitable medium is chosen for growing an appropriate micro-organism. The method has been adapted to amino-acids, of which no less than 16 can now be assayed. An important advantage of this type of method is that it can satisfactorily be applied to such mixtures as leucine with isoleucine, which cannot conveniently be separated chemically. Penicillin and chromatography are both very topical, and C. R. Bond brought them together in discussing the assay of penicillin. As produced by moulds, penicillin is a mixture of at least 10 acids with similar general structure, but different side chains. It becomes important, therefore, to determine not only total potency—as by the plate or turbidimetric methods—but also the proportions of the different components. Many methods have been proposed for the latter purpose, and use of micro-chromatography on buffered filter-paper strips has been described by Goodall and Levi. After treatment, a strip is mounted on an inoculated agar plate, and zones of inhibition due to the individual penicillins can be observed after incubation; quantitative data can be derived from the zones. In the last paper, Mr. A. Parker spoke of aseptic technique in industrial fermentations, a topic of special importance in connection with penicillin manufacture; a number of principles have guided the design and operation of plant. Thus, seed cultures must be prepared in such a way as to guard against all possibility of contamination, and the plant itself must be susceptible of complete sterilization and must exclude all possible contaminants. Sterile air must be supplied

and every stage of the process must be carefully checked, whilst plant operation must be of such kind that it can be carried out efficiently by relatively unskilled labour. On these lines, considerable success has been achieved, enabling a close approach to be made to the ideal pure culture system.

It was a happy arrangement that the Friday and Saturday of the Symposium week should have been devoted to meetings of the Bio-chemical and Physiological Societies, held at Dundee and St. Andrews respectively. Members of the various organizations involved were thus enabled to get together both formally and informally to their mutual advantage. Social events also were arranged throughout the week, and included a formal dinner, conducted tours of St. Andrews itself, a tour of the Fife coast, a visit to Falkland Palace, and a visit to a well-known brewery. Great credit is due to all those responsible for the details of arrangements made both on the business and social sides, and special mention may perhaps be made of Dr. D. Traill (Chairman of the Symposium Committee) and Mr. A. R. Jamieson (Hon. Secretary), whilst the facilities provided by Principal Sir James Irvine and the Staff of the Chemistry Department of the University earned the sincere appreciation of all who attended. It is a matter of great satisfaction that so large a gathering was attracted to this function; it is seldom that so comprehensive an opportunity is afforded of appreciating what the term "Fermentation Industries" really implies. The wide scope of the lectures, and the opportunities of discussion provided after the lectures, would appear to have met a real need in bringing together and considering a mass of useful information which is not readily accessible in the usual literature. It is to be hoped that it will prove possible to publish the full proceedings, to be available for student use and for general reading alike.

* * * *

THE 1949 HOP CROP

BY SYDNEY MYER

THE annual caution, that cannot be too strongly emphasized, is that one can only write of what one sees, and conclusions now prompted are always subject to the atmospheric conditions that August—the all-important period—provides. An experience of over 60 years would have suggested that this crop, grown under long drought

and high temperatures, must show definite signs of lack of moisture; yet a special journey to the Kent district made this week does not bear this out, and there is hardly any indication—such as yellowing of the bine, or "tiredness"—that has previously been noticeable under similar dry conditions. It has been suggested that this is accounted for by the fact that the drought is not merely a summer one, but dates back to last autumn, so that the roots instead of developing at normal winter level had to throw out lower suckers, tapping deeper strata of soil; these have continued to find moisture for the plant. This does seem a feasible explanation.

There have been the usual visitations of aphid and red spider, but both have been mainly overcome by intensive powdering and washing. With regard to the method of attacking these pests, experiments are being made with much more powerful washes and powders—so strong, indeed, that instructions are issued to men carrying out the operation reminiscent of advice given during the war in the event of phosgene gas being used, and the importance of not allowing it to come into contact with the skin! There is no doubt of the complete destruction of both aphid and spider, but whether there is any deleterious effect on the plant, or any drawback from cumulative effect, is not yet assured. One feature noticeable as a result of using stronger insecticides is the rareness of the lady-bird (and in its previous stage, the "nigger"), which used in days prior to washing machines to be Nature's antidote to aphid. The western hop district has had more rain than the southern, even if well below normal, and is looking quite promising. In trying to suggest a general view of the crop at this juncture, it certainly can be said that it looks surprisingly well—the burr is strong and healthy, and the laterals give reason to anticipate a crop of full average size. This conclusion is influenced by the fact that one-third of the total acreage has been planted in the last few years, and one can always expect especial vigour from a new hop plant. It is naturally impossible at this stage to say anything with regard to quality, but there is every likelihood that it will be much superior to last season's, a view the writer hopes to be able to amplify later in the year.

28th July, 1949.

THE INSTITUTE OF BREWING EXAMINATIONS: JUNE, 1949

PASS LIST

FIRST ASSOCIATESHIP EXAMINATION

(42 entered; 27 passed)

AGER, Anthony Robert, Hertford.
 BINNS, George, Winster, Derbyshire.
 BLACKER, Fred Eric, Edinburgh.
 CALDERBANK, Thomas Geoffrey, Bolton.
 CHRISTIAN, Denys Alex Keith, Edinburgh.
 CLIFF, Patrick Bertram, Truro.
 COX, Edwin Grant, Bristol.
 DUTTON, Edward Piers de Charriere, Chesterfield.
 DYKES, James Edward Townley, Edinburgh.
 *FALCON, Michael Gascoigne, Norwich.
 HERON, John Robert, Northampton.
 HOGG, John Laird, Edinburgh.
 ILLINGWORTH, Douglas Kenneth, New Basford.
 *KERR, Archibald James, Edinburgh.
 LITTLETON, Leslie Derek Cooper, West Kirby.
 MANN, Peter Dalla, Edinburgh.
 MAULE, Antony Carteret, Sheffield.
 NIMMO, Robert Walter, Perth.
 SHEARD, John Neville, Manchester.
 STEVENSON, Alexander, Edinburgh.
 STOCKAN, Ronald James, Edinburgh.
 STRICKLAND, JOHN Addington Stott, Northampton.
 THOMSON, Ronald Matthew, Edinburgh.
 WALL, Alan Desmond, Mansfield Woodhouse, Notts.
 WATTS, Eric John, Blackburn.
 WEARE, Roger Gerald, Tunbridge Wells.
 WHITE, Victor Stanley, Edinburgh.

* "With Distinction."

SECOND ASSOCIATESHIP EXAMINATION

(24 entered; 17 passed)

BARBOUR, Thomas Downie, Dunbar.
 *BAXTER, William Dick, Edinburgh.
 *BROWN, James Edward, Burslem.

CHRISTIAN, Denys Alex Keith, Edinburgh.
 CLARK, John, Southwold.
 DYKES, James Edward Townley, Edinburgh.
 FERGUSON, Thomas Crawford, Edinburgh.
 FULLERTON, Lionel Mackay, Edinburgh.
 HARPER, Norman William, Highbury, N.5.
 *HENSON, John Carter, Liverpool.
 LITTLETON, Leslie Derek Cooper, Birmingham.
 McDONALD, Eric Young, Edinburgh.
 MEPHIUS, Ronald George Lund, Edinburgh.
 *STEPHENSON, Peter Dixon, Ripon.
 WALL, Alan Desmond, Mansfield Woodhouse, Notts.
 WALLIS, Anthony James, London.
 WOODLAND, Raymond, Taunton.

* "With Distinction."

DIPLOMA MEMBERSHIP EXAMINATION

(13 entered; 13 passed)

*ABBOTT, Howard, Burton-on-Trent.
 *BRADBURY, John Harvey, B.Sc.Tech.(Man)., A.M.C.T., Wellington.
 BRUCE, James Marshall Cameron, Abertillery, Mon.
 CLANCY, James Paul Ivan, B.A.(Queen's Univ.), Ottawa, Ontario, Canada.
 DAVIS, John Ralph, B.S.A.(Ontario), Ottawa, Ontario, Canada.
 *DONALD, Malcolm Pringle, Bolton.
 *GUEST, John Holden Elliot, B.A.(Cantab.), Burton-on-Trent.
 *JESSUP, George Albert, Maidstone.
 KAVANAGH, James Joseph, B.A.(Toronto), Toronto, Canada.
 MONCKTON, Herbert Anthony, Luton.
 MORGAN, Eric John, Burton-on-Trent.
 *PENROSE, John Denis Fitz-Gerald, Milton-under-Wychwood, Oxon.
 *WILLIAMSON, Alan Graham, B.A.(Toronto), Weston, Ontario, Canada.

* "With Honours."

THE INSTITUTE OF BREWING RESEARCH SCHEME

NITROGEN METABOLISM OF YEAST. A CONSIDERATION OF THE
MODE OF ASSIMILATION OF AMINO ACIDS

By R. S. W. THORNE, Ph.D.

Yeast growth is much more active on wort than when nitrogen is supplied as an ammonium salt which, in turn, will in general support growth better than do individual amino acids. These facts cannot be explained in terms of the Ehrlich and Stickland mechanisms of deamination, and it is suggested that a third mechanism is involved, namely, that when yeast is supplied with a complex mixture of amino acids it tends to assimilate them intact, with more rapid utilization than if deamination were involved. If a necessary amino acid is lacking, deamination will be required to allow its synthesis from ammonia. The hypothesis is supported by the enhanced values of amino acid mixtures as compared with single amino acids, and by the high rate of growth on casein hydrolysate accompanied by greater availability of sugar for fermentation. Also, the effects of deficiency of pyridoxin (presumed to be involved in deamination) are less marked with the hydrolysate than with single amino acids.

SECTION I

*The Classical Theory of Nitrogen Assimilation
from Amino Acids.*

Modern knowledge of the nitrogen nutrition of yeast is rooted in the work of Ehrlich. Ehrlich's initial interest was to trace the origin of the amyl alcohols which arise during fermentation, and he succeeded in proving that they are formed from the leucines present in the unfermented nutrient medium. For example, by adding a heavy seeding of yeast to a solution of sugar and leucine and fermenting away all the sugar (*Ber.*, 1907, 40, 1027) he obtained an 80 per cent. yield of isoamyl alcohol from the leucine which had been metabolized by the yeast. This is consistent with a hydrolytic deamination and decarboxylation of the leucine:

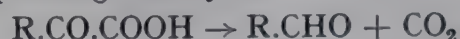


Exactly similar reactions were found by Ehrlich to occur with isoleucine (*loc. cit.*), phenylalanine, tyrosine (*ibid.*, 1911, 44, 139) and tryptophane (*ibid.*, 1912, 45, 883) and at a later date by the present writer with valine (this *Journ.*, 1937, 288). The apparently anomalous origin of succinic acid from glutamic acid was also established by Ehrlich (*Biochem. Z.*, 1909, 18, 391). The general mechanism of these changes undergone by

amino acids was worked out by Neubauer and Fromherz (*Z. physiol. Chem.*, 1911, 70, 326) and Neuberg and Hildesheimer (*Biochem. Z.*, 1911, 31, 170). The initial reaction is an oxidative deamination leading to the formation of a ketonic acid:



This is followed by the decarboxylation of the ketonic acid with formation of the corresponding aldehyde:



Finally the aldehyde may be reduced to an alcohol, as in the production of isoamyl alcohol from leucine:



or it may be oxidized to an acid, as happens when succinic acid is produced from glutamic acid:



The ammonia which is liberated by these reactions is utilized by the yeast as fast as it is formed, for it does not appear in the fermenting medium at all. It is important to note that cell-free extracts of yeast are unable to carry out these reactions; indeed, not only living, but also actively growing yeast is necessary, since they do not even occur with living yeast in the absence of

fermentable sugar. It is thus evident that the deamination of α -amino acids is bound up with the life of the yeast cell since it provides the nitrogen indispensable for growth and multiplication.

Because the nitrogen in, for example, beer wort consists largely of protein degradation products of which the α -amino acids are especially significant it has been generally assumed, on the strength of Ehrlich's work, that these amino acids are simply deaminated in the way that he discovered, the resulting ammonia being used as the essential raw material for the elaboration of new yeast protein and other necessary nitrogenous materials. This view leads to certain difficulties which will be discussed below. But it is worth pointing out at once that the experimental basis of Ehrlich's work does not offer a strictly valid ground for the above inference. The fact that several amino acids when presented individually to yeast as the sole source of nitrogen undergo the type of deamination now known as the *Ehrlich mechanism* is no proof that the same mechanism operates exclusively, nor even primarily, when a whole array of amino acids is presented simultaneously to yeast. On the other hand, the production of fusel oil (a collective term for the higher alcohols formed from amino acids by the Ehrlich mechanism) in such fermentation products as beer demonstrates that a considerable proportion of the amino acids must be so deaminated. Ehrlich showed also that the addition of ammonia to a fermenting nutrient medium depresses the production of fusel oil demonstrating thereby that yeast assimilates ready-formed ammonia in preference to deaminating amino acids.

The belief that nitrogen is assimilated by yeast from the amino acids in wort in accordance with the Ehrlich mechanism naturally focussed attention on the nutrient value of the individual amino acids. An obvious corollary of the Ehrlich view is that if the amino acids function as nutrients solely by supplying ammonia to the yeast, the nutrient value of ammonia should represent an upper limit which could be exceeded by no amino acid. So long as consideration is confined to single amino acids this is largely true. Work on the nutrient value of single amino acids in comparison with ammonium phosphate was described by the present writer (this *Journ.*, 1941, 255), measurements being

made of the fermentation, nitrogen uptake and rate and extent of yeast growth of a top-fermentation strain of *S. cerevisiae* (N.C.T.C., No. 6479) when various amino acids were supplied as the sole source of nitrogen in a synthetic nutrient medium. Since the growth characteristics mentioned run more or less parallel with each other it is convenient for the present purpose to average them all and express them on an arbitrary scale of nutrient value, the value for ammonium phosphate being set at 100. This is done in Table I. It is seen that of the 18 amino acids examined glutamic acid

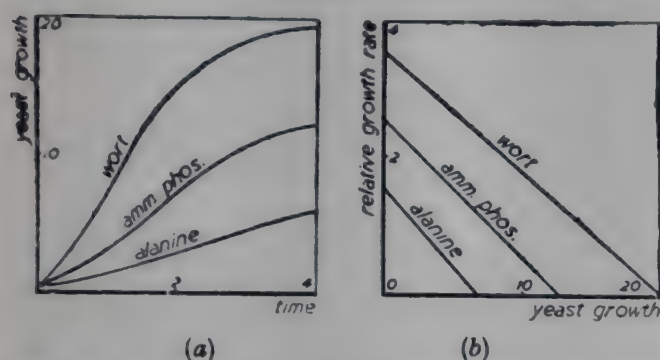
TABLE I
RELATIVE NUTRIENT VALUES OF DIFFERENT
NITROGEN SOURCES
(Based on data given in this *Journ.*, 1941, 255)

	Relative nutrient value.
Ammonium phosphate ..	100
Glycine	15
Alanine	68
Serine	59
Valine	78
Leucine	87
isoLeucine	65
Aspartic acid	128
Glutamic acid	104
Lysine	8
Cystine	22
Methionine	64
Arginine	87
Phenylalanine	64
Tyrosine	58
Proline	69
Hydroxyproline	42
Histidine	31
Tryptophane	49
Asparagine	142
(Worts)	150-200

is practically identical in nutrient value to ammonium phosphate, while only aspartic acid is superior. The remaining 16 amino acids vary over a wide range. The insignificant nutrient value of glycine, lysine and cystine is especially noteworthy; under the conditions of these experiments they are not in fact deaminated at all. The average nutrient value of all the amino acids is 61, or approximately two-thirds that of ammonium phosphate. The anomalous results for aspartic acid and its amide, asparagine, will be discussed later. The spread of these data in terms of growth curves is illustrated in Diagram I. I (a) shows curves of yeast

growth (grms. moist yeast *per* litre) against time (days) for a typical wort, ammonium phosphate, and an amino acid—alanine—whose characteristics are near the average for the class. I (b) shows the corresponding relations between yeast growth and the relative growth rate (see Thorne, *ibid.*, 1939, 472).

DIAGRAM I



Range of variation of yeast growth with different nitrogen sources

It will be observed that the nutrient value of wort is strikingly greater than that of ammonium phosphate and still more so in comparison with individual amino acids. Since the principal source of assimilable nitrogen in wort consists in fact of amino acids the superior growth of yeast in wort is paradoxical so long as the Ehrlich mechanism is held to be the only one operative. In the early days of work with synthetic nutrient media it was possible to believe that these were defective with respect to some growth factor or factors, the superiority of wort being due to its containing an abundance of these factors. It will be shown that this explanation is now untenable.

It is important to discover why the amino acids listed in Table I vary so much among themselves with respect to their nutrient value since they all function in the same way by supplying ammonia to the yeast. Two possibilities suggest themselves: (i) that the residues left after their deamination have varying toxicities towards yeast, and (ii) that they vary in the readiness with which they undergo deamination. The present writer (*ibid.*, 1939, 13) has determined the toxicities of a number of the amino acid residues and expressed them in terms of inhibition factors which are a measure of the extent to which growth and fermentation are slowed down by any particular concentration of a particular inhibitor. (An inhibition factor of 0

implies no inhibition, while 1 implies the complete arrest of growth or fermentation.) In the last column of Table II are recorded

TABLE II
RELATIONSHIP BETWEEN INHIBITOR PRODUCTION
AND YEAST GROWTH

(Based on data given in this *Journ.*, 1939, 13 and 1941, 255)

	Growth inhibition factor, <i>k</i> , calculated from nitrogen assimilated.	Yeast growth, <i>Y</i> , in grms. <i>per</i> litre.
Alanine	0.01	7.3
α-Aminobutyric acid	0.12	8.0
Valine	0.03	8.6
Leucine	0.06	12.1
isoLeucine	0.10	6.8
Glutamic acid	0.00	12.7
Ornithine	0.00	9.4
Arginine	0.00	9.8
Phenylalanine	0.09	7.3
Tyrosine	0.00	7.1
Tryptophane	0.16	3.7

the final yeast growths, *Y*, attained when yeast 6479 is grown with various amino acids as the sole source of nitrogen (*ibid.*, 1941, 255). Measurements made at the same time of nitrogen assimilated enable the amounts of amino acids deaminated to be inferred and hence the amounts of inhibitor produced; the previously determined toxicities of these inhibitors in conjunction with their calculated concentrations enable the growth inhibition factors, *k*, to be estimated. These are given in the second column of Table II. It is not easy to see at a glance what correlation exists between these two variables, except that it is clearly not complete. The correlation may, however, be estimated precisely by calculation of the correlation coefficient, *r*. (The standard formula applied to this particular case is:

$$r = \frac{\sum(k - \bar{k})(Y - \bar{Y})}{\sqrt{\sum(k - \bar{k})^2 \cdot \sum(Y - \bar{Y})^2}}$$

where $\bar{k}$ and $\bar{Y}$ signify the mean values of *k* and *Y* respectively in the sample.) The value obtained is *r* = -0.59 which, being negative, implies an inverse correlation between the variables, *i.e.* as *k* increases so *Y* tends to diminish. Standard tables of the significance to be attached to the correlation coefficient show that there is only a chance

of 1 in 20 that this value of r could arise fortuitously; the correlation between k and Y should therefore be accepted as significant. The square of the correlation coefficient is a quantity known as the *explanation*, a measure in this particular case of the proportion of the variability of the yeast growth which can be explained as due to variation in the growth inhibition factor. The explanation here is given by $-0.59^2 = 0.35$; that is to say, approximately one-third of the effect can be attributed to inhibitor production. It may therefore be concluded from the data of Table II that (i) there is a real correlation between yeast growth and production of inhibitors, one-third of the total variability being attributable to this, and (ii) the remaining two-thirds of the variability must be attributed to variation in the ease with which different amino acids undergo deamination.

This brief survey of the Ehrlich theory of nitrogen assimilation by yeast may thus be summarized as follows:—

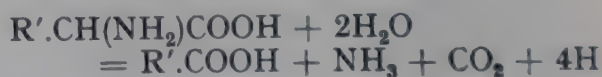
- (i) The majority of amino acids when presented singly to yeast as sources of nitrogen undergo deamination according to the Ehrlich mechanism and, in consequence, are more or less inferior to ammonia in nutrient value. Variations amongst them in this respect are attributable mainly to differences in their ease of deamination, but partly also to differences in the toxicities of their by-products.
- (ii) A few amino acids appear to resist deamination and are useless as nitrogen sources.
- (iii) Aspartic acid alone among amino acids shows a significantly higher nutrient value than ammonia so that the Ehrlich mechanism seems inadequate to explain its behaviour. Its amide, asparagine, is also superior to ammonia.
- (iv) Fusel oil is produced in wort during its fermentation, indicating that the Ehrlich mechanism must be operating; the strikingly superior growth of yeast in wort compared with its growth in single amino acids suggests, however, that the Ehrlich mechanism can hardly be the principal one.

SECTION 2

Yeast Growth in Binary Mixtures of Nitrogen Nutrients.

From the foregoing discussion it seems logical to seek an explanation of the superiority of wort in the fact of its containing a mixture of nitrogen nutrients. As a step towards unravelling the problem the writer has recently published data on the nutrient value of various binary mixtures of nitrogen sources (*ibid.*, 1944, 186; 1946, 5, 15). The data cited here are for mixtures containing equal contributions of assimilable nitrogen from each of the two sources. In almost all the cases examined there is an enhancement of fermentation, nitrogen uptake and yeast growth and growth rate compared with the mean values for the two nutrients used separately. The enhancements found are given in the third column of Table III and are expressed as the percentage improvement of nutrient value in the binary mixture over the mean nutrient value of its components. Before commenting on these results it will be necessary to give a brief account of a type of interaction between amino acids which was discovered by Stickland (*Biochem. J.*, 1934, 28, 1746; 1935, 29, 288, 889).

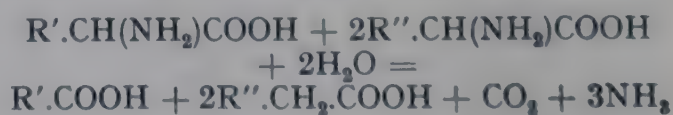
Stickland was studying the metabolism of the anaerobic bacterium *Clostridium sporogenes*. This organism lacks normal oxidative mechanisms and was found to obtain its energy by oxidizing some of the amino acids present in the medium at the expense of others. The amino acids were found to be classifiable into two groups, namely, those which can act as hydrogen donors:



and those which can act as hydrogen acceptors:



The net reaction which occurs between amino acids of the two classes is therefore:



one amino acid being oxidized by the other with the liberation of ammonia. The

TABLE III

PERCENTAGE ENHANCEMENT OF YEAST GROWTH AND FERMENTATION IN BINARY MIXTURES OF VARIOUS NITROGEN NUTRIENTS

(Based on data given in this *Journ.*, 1944, 186; 1946, 5, 15)

	Stickland Interaction.	Enhancement.	Mean Enhancement.
Ammonium phosphate-asparagine ..	—	4	20
Ammonium phosphate-aspartic acid ..	—	10	
Ammonium phosphate-glutamic acid ..	—	6	
Ammonium phosphate-leucine ..	—	10	
Ammonium phosphate-arginine ..	—	14	
Ammonium phosphate-histidine ..	—	40	
Ammonium phosphate-glycine ..	—	62	
Ammonium phosphate-alanine ..	—	20	
Ammonium phosphate-tyrosine ..	—	11	14
Asparagine-glycine	?	49	
Asparagine-glutamic acid	?	10	
Asparagine-arginine	?	3	
Tyrosine-glycine	?	-8	8
Aspartic acid-alanine	—	19	
Leucine-alanine	—	6	
Leucine-aspartic acid	—	9	
Alanine-valine	—	-6	
Valine-leucine	—	12	32
Leucine-glycine	+	43	
(Alanine-proline	+	4)	
Alanine-glycine	+	94	
Histidine-glycine	+	20	
Phenylalanine-glycine	+	29	
Arginine-leucine	+	14	
Arginine-aspartic acid	+	7	
Arginine-glutamic acid	+	18	

15 amino acids examined by Stickland were classifiable as follows:—

Hydrogen donators: alanine, serine, valine, leucine, aspartic acid, glutamic acid, cysteine, phenylalanine, histidine;

Hydrogen acceptors: glycine, ornithine, arginine, proline, hydroxyproline, tryptophane.

Evidence has been provided by the present writer (this *Journ.*, 1944, 186) that yeast also is able to initiate the Stickland reaction given suitable pairs of amino acids. This reaction is significant in the case of yeast, however, not as a source of energy but as a source of ammonia additional to that made available by the Ehrlich mechanism. In this sense it will be referred to as the *Stickland mechanism*.

Returning now to Table III it will be seen that the binary mixtures studied fall into four groups, namely, (i) the cases in which

ammonium phosphate is one constituent and in which, of course, the Stickland mechanism cannot operate, (ii) the cases where it is unknown whether or not the Stickland mechanism operates, (iii) the cases where the Stickland mechanism cannot operate because both constituents fall into the same class of amino acids, and (iv) the cases where the Stickland mechanism can operate because the constituent amino acids belong to opposite classes.

Of the 26 binary mixtures studied only two fail to give a positive enhancement of yeast growth and fermentation. It may, therefore, be presumed that although occasional antagonisms may occur among the amino acids, the admixture of nitrogen nutrients is, as a rule, distinctly beneficial. In the group of mixtures where the Stickland mechanism is possible the enhancements obtained are, in accordance with expectation, on the whole the greatest. The average enhancement for this group is 32 per cent.,

which must be largely attributed to extra ammonia made available by the Stickland mechanism. (The alanine-proline mixture is omitted from this mean as it happens that proline is exceptional among the hydrogen-accepting class of amino acids: it undergoes reduction not by deamination but by ring-fission and, consequently, does not furnish the yeast with any extra ammonia.) It is next to be noticed that even the group of amino acid mixtures in which the Stickland mechanism cannot operate nevertheless gives an appreciable enhancement though its magnitude is only one-quarter that of the previous group. This is a very suggestive fact, implying that factors other than the Ehrlich and Stickland mechanisms need to be considered in studying yeast growth in wort.

Of the Stickland mechanism it is therefore to be concluded that it provides an adequate explanation of the much greater enhancements obtained in some binary mixtures of amino acids than in others, but it provides no explanation at all of the smaller enhancements that are obtained in mixtures where its occurrence seems to be precluded. There is every reason to suppose that it occurs during yeast growth in wort, for the conditions there would favour it, but as it furnishes merely an alternative or additional supply of ammonia to that produced by the Ehrlich mechanism it can contribute nothing whatever towards resolution of the paradox that yeast growth in wort is very much better than that in ammonia. (A tentative calculation can be made of the improvement in wort growth which might be expected to arise from the operation of the Stickland mechanism: of the 15 amino acids studied by Stickland nine are hydrogen donors and five (excluding proline) hydrogen-acceptors; $15 \times 14 = 210$ possible pairs come into consideration, of which only $9 \times 5 = 45$ can initiate the Stickland mechanism. The average relative nutrient value of the single amino acids is 61, while the average enhancement produced by the Stickland mechanism is 32 *per cent.*; therefore, taking account of both Ehrlich and Stickland mechanisms, the nutrient value of a mixture of these 15 amino acids should be:

$$61 \times (45/210) \times (132/100) + 61 \\ \times (210 - 45)/210 = 65$$

Thus the nutrient value will be increased

from 61 to 65, an enhancement of 7 *per cent.* The limitations of this calculation are obvious, but it serves to show that Stickland enhancements can account for very little of the superiority of yeast growth in wort.)

The principal points emerging from the study of yeast growth in binary mixtures are:

- (i) Enhancements of yeast growth and fermentation are commonly encountered when nutrients are mixed; antagonisms between amino acids may also occur, but are rare.
- (ii) The very considerable enhancements which occur with certain pairs of amino acids have disclosed the existence of a second mechanism of nitrogen assimilation, the Stickland mechanism, which like that of Ehrlich, furnishes ammonia to the yeast, but leaves acidic residues behind in the fermented medium.
- (iii) After account has been taken of the Stickland mechanism there are still unexplained residual enhancements given by binary mixtures and, since the magnitude of the Stickland enhancements to be expected in wort is not large, these remaining enhancements may perhaps provide the clue to the problem of yeast growth in wort.

SECTION 3

The Assimilation of Nitrogen from Wort

According to the Ehrlich theory, coupled with the determination of the nutrient value of individual amino acids made by the present writer, it is to be expected that if nitrogen in the form of ammonium phosphate were added to wort it should be assimilated by yeast more rapidly than the wort nitrogen itself. This experimental device of adding ammonia to the wort has an important advantage over parallel observations of yeast growth in wort and in synthetic media since it automatically compensates for uncontrollably different characteristics of the media: in particular, any superiority of wort with respect to growth factors is shared equally by ammonium phosphate when it is mixed in with the wort. Consequently, although separate observations of growth in wort and in ammonium phosphate synthetic

TABLE IV

ASSIMILATION OF TOTAL AND AMMONIA NITROGEN FROM A WORT CONTAINING ADDED AMMONIUM PHOSPHATE
(All quantities expressed in grms. *per* litre)

Yeast growth (moist).	Uptake of total nitrogen.	Uptake of ammonia nitrogen.	Uptake of non-ammonia nitrogen.	Uptake of non-ammonia nitrogen corrected for excretion.
1.4	0.047	0.002	0.045	0.047
2.4	0.078	0.010	0.068	0.072
4.3	0.114	0.029	0.085	0.092
4.6	0.113	0.037	0.076	0.084
5.8	0.137	0.042	0.095	0.105
5.8	0.152	0.057	0.095	0.106
7.9	0.195	0.078	0.117	0.136
10.4	0.221	0.097	0.124	0.151

media show a marked superiority of the former, it may be anticipated that with the new technique ammonium phosphate would be able to show in actual practice its theoretical superiority as a nutrient to amino acids. It must be appreciated that in this type of experiment although ammonia will function as a nutrient, it is being added not for this purpose specifically, but in order to act as a reproducible standard of comparison for assessing the rate of assimilation of wort nitrogen. This method of estimating assimilation rates of non-ammonia nitrogen in the presence of ammonia is obviously only valid provided the yeast does not excrete ammonia into the medium during its growth. Experiments carried out to test this point have shown that no measurable amount of ammonia is produced in wort during yeast growth; the small amount of ammonia normally present in wort is in fact taken up by the yeast. In anticipation of later sections of this paper it may also be mentioned that no ammonia production in the medium is observable when yeast is grown with asparagine as the nitrogen source, nor with casein hydrolysate.

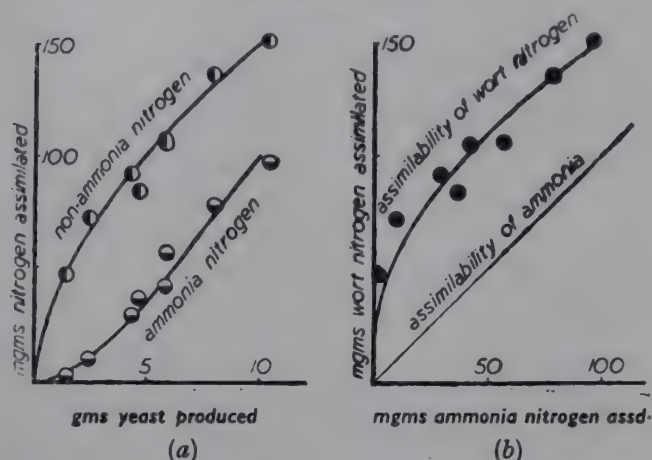
The result of such an experiment is given in Table IV. The nutrient medium consisted of a hopped wort of specific gravity 1.045, diluted with an equal volume of standard mineral salt solution (1,000 ml. distilled water; 2 grms. potassium dihydrogen phosphate; 1 gm. magnesium sulphate, cryst.; 0.2 gm. calcium sulphate; 0.01 gm. ferrous sulphate); the specific gravity of the diluted wort was raised to 1.029 by the addition of 20 grms. of sucrose *per* litre; 0.71 gm. of

ammonium phosphate *per* litre was then added. The total nitrogen content of this medium was 0.538 gm. *per* litre, of which 0.156 was ammonia nitrogen; the concentration of non-ammonia or wort nitrogen was therefore 0.382 gm. *per* litre. It may be assumed that about one-half the total nitrogen of wort is assimilable, so that in this experiment the concentration of ammonia nitrogen was somewhat less than that of the assimilable wort nitrogen. The nutrient medium was then set out in 100 ml. portions which were inoculated with varying amounts of yeast and allowed to grow with constant shaking at 21°C. After various time intervals (18, 24 and 42 hours) the amounts of yeast growth were estimated turbidimetrically. Those flasks in which the yeast had grown to a suitable extent were withdrawn, the yeast removed by filtration and determinations made of the total nitrogen (Kjeldahl) and ammonia nitrogen (distillation with magnesia) in the partially fermented media.

Table IV shows, for varying amounts of yeast growth, the amounts of total nitrogen and ammonia assimilated; the difference between these two gives the apparent amount of non-ammonia nitrogen assimilated. As shown in an earlier paper by the writer (*ibid.*, 1945, 6) this non-ammonia nitrogen is too small by an amount equal to the quantity of nitrogen excreted by the yeast; a method was there given for correcting for this factor. The results of applying these corrections, which are small for the earlier stage of yeast growth, but which become considerable towards its end, are given in the last column

of Table IV. The trend of these experimental results is shown clearly in Diagram II. In II (a) are given the curves obtained by plotting ammonia and non-ammonia assimilation against yeast growth. It is immediately apparent that ammonia nitrogen instead of being assimilated at a greater rate than

DIAGRAM II



Uptake of ammonia and non-ammonia nitrogen from wort containing added ammonium phosphate

non-ammonia nitrogen, as Ehrlich's theory requires, is assimilated much more slowly, at least during the earlier stage of yeast growth. II (b) gives the curve obtained by plotting non-ammonia or wort nitrogen

uptake against ammonia nitrogen uptake, and shows the disparity between these two still more clearly. Since the two variables are plotted on the same scale, the assimilability of ammonia, for comparison with the wort nitrogen assimilability curve, is represented by a straight line with a slope of 45° passing through the origin. Approximately the first 100 mgrms. of the wort nitrogen have been assimilated more rapidly than ammonia.

The implications of this simple experiment are of such obviously fundamental importance in relation to current ideas about assimilation of nitrogen by yeast that it has been repeated many times, always giving essentially the same result. Table V gives a condensed summary of the experimental data obtained by the above technique from nine different mixtures of wort and ammonium phosphate. (The details of these mixtures are as follows: (I) hopped wort, sp.gr. 1.035, undiluted but decolourized with charcoal, a treatment which also removes some of the nitrogen; (II) hopped wort, sp.gr. 1.035, undiluted and partially decolourized with charcoal; (III) hopped wort, sp.gr. 1.040, diluted with equal volume of standard mineral salt solution and sp.gr. raised to 1.034 by addition of sucrose; (IV) sweet wort,

TABLE V

ASSIMILATION OF NON-AMMONIA AND AMMONIA NITROGEN FROM NINE WORTS CONTAINING ADDED AMMONIUM PHOSPHATE

(All quantities expressed in grms. per litre)

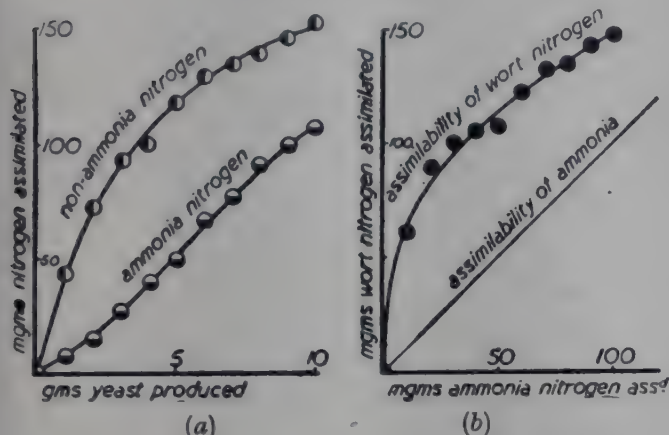
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	Means.	Means corrected for concentration.
(a)*	0.364	0.441	0.552	0.268	0.433	0.382	0.373	0.393	0.395	0.400	
(b)†	0.200	0.235	0.176	0.155	0.158	0.156	0.433	0.155	0.437	0.234	
Ammonia nitrogen uptake.	Non-ammonia nitrogen uptake.										
0.010	0.024	0.055	0.091	0.045	0.088	0.086	0.028	0.072	0.067	0.062	0.063
0.020	0.045	0.078	0.136	0.062	0.119	0.124	0.044	0.084	0.115	0.090	0.091
0.030	0.055	0.081	0.176	0.079	0.147	0.096	0.058	0.091	0.122	0.101	0.103
0.040	0.064	0.085	0.204	0.090	0.159	0.091	0.059	0.097	0.103	0.106	0.108
0.050	0.068	0.091	0.215	0.097	0.153	0.098	0.060	0.106	0.084	0.108	0.110
0.060	0.080	0.101	0.226	0.104	0.169	0.113	0.083	0.110	0.133	0.124	0.126
0.070	0.088	0.132	0.236	0.110	0.181	0.127	0.102	0.127	0.101	0.134	0.136
0.080	0.092	0.147	0.237	0.116	0.177	0.144	0.117	0.138	0.092	0.136	0.138
0.090	0.095	0.146	0.243	0.112	0.173	0.157	0.127	0.145	0.093	0.144	0.146
0.100	0.099	0.142	0.253	0.118	0.187	0.167	0.129	0.158	0.093	0.150	0.152
standard deviation											± 0.014

* (a) = Initial non-ammonia nitrogen.

† (b) = Initial ammonia nitrogen.

sp.gr. 1.032, diluted with equal volume of standard salt solution and sp.gr. raised to 1.029 by addition of sucrose; (V) hopped wort, sp.gr. 1.046, diluted with equal volume of standard mineral salt solution and sp.gr. raised to 1.034 by addition of sucrose; (VI and VII) sweet wort, sp.gr. 1.045, diluted with equal volume of standard mineral salt solution and sp.gr. raised to 1.029 by addition of sucrose; (VIII and IX) hopped wort,

DIAGRAM III



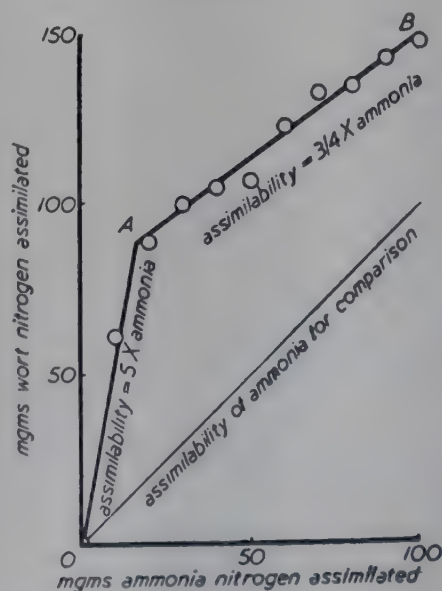
(a) Averaged curves of non-ammonia and ammonia nitrogen uptake from worts containing added ammonium phosphate

sp.gr. 1.040, diluted with equal volume of standard mineral salt solution and sp.gr. raised to 1.027 by addition of sucrose. These worts were diluted in order to reduce their nitrogen contents to a convenient level; experiments with undiluted worts, which will be quoted below, give results concordant with these). The first two lines of Table V give the initial concentrations of non-ammonia and ammonia nitrogen in these media. The experimental results obtained were plotted as in Diagram II (b), successive points being joined by straight lines; the amounts of non-ammonia nitrogen assimilated for values of 0.010, 0.020 . . . 0.100 grms. ammonia nitrogen assimilated were then read off by interpolation. It is these values which are recorded in the second to tenth columns of Table V. The last column but one in this table gives the mean values of wort nitrogen assimilated for each successive level of ammonia assimilation. The average initial wort nitrogen concentration is 0.400 gm. per litre, of which one-half, 0.200, may be regarded as assimilable; the initial ammonia nitrogen concentration, 0.234, is a little greater than this. A correction (*loc. cit.*) can

be applied to give the result for equal concentrations of the two forms of nitrogen; this has been done in the last column of the table, the correction, it will be seen, being very small. These final experimental data are shown graphically in Diagram III (b), the corresponding separate curves for wort nitrogen uptake and ammonia nitrogen uptake against yeast growth being given in III (a).

The average result for these nine worts amply confirms the conclusion already drawn that wort nitrogen, or rather, a large proportion of it, is far more assimilable than ammonia nitrogen. It should be pointed out that there is a considerable scatter of the results for individual worts about the mean values recorded in the last column of Table V and this is reflected in the relatively large value of the standard deviation, ± 0.014 ; the difference between the rate of uptake of wort nitrogen and ammonia nitrogen lies far outside the range of this standard deviation, however, and is overwhelmingly significant. Apart from observational error the variability of the individual results may be due to uncontrolled variations in the worts and probably to variations in the response of the yeast at different times.

DIAGRAM IV



Analysis of wort nitrogen assimilability curve

The relationship between wort nitrogen and assimilation instead of being represented by a smooth curve drawn through the points as in Diagram III (b) can be represented almost equally well, and certainly more

illuminatingly, by two straight lines as shown by the lines OA and AB in Diagram IV. The possibility of this representation suggests that there are two fairly distinct elements to be considered in wort nitrogen. The slope and height of the line OA indicates that over half of the assimilable wort nitrogen is taken up at no less than *five times* the rate of assimilation of ammonia nitrogen. When this element of the wort nitrogen, whatever it may be, is all consumed there is a sudden drop in the rate of assimilation of the remaining wort nitrogen as represented by the line AB, the slope of which indicates a rate of assimilation *less* than that of ammonia. The actual value appears to be three-quarters of the rate of ammonia assimilation, which is rather close to the average rate found for individual amino acids.

The possible nature of the very highly assimilable element in wort nitrogen must now be considered. The approximate distribution of the different forms of nitrogen in wort, expressed as 'percentages' of the permanently soluble nitrogen is, according to Schryver and Thomas (*ibid.*, 1929, 571):—

Ammonia nitrogen	..	..	3
Amide nitrogen	..	..	8
Amino nitrogen	..	..	18
Peptide nitrogen	..	..	28
Undetermined nitrogen	..	..	43

The ammonia fraction is obviously of no relevance to the problem under discussion and is, in any case, much too small in amount to affect the issue. The amino nitrogen seems at first sight to be excluded on account of the relatively low nutrient value of the individual amino acids. The peptide nitrogen must also be excluded because experiments by Damlé and Thorne (*ibid.*, 1949, 13) have shown that although the nutrient value of simple peptides may not be much lower than that of amino acids, it is certainly not higher; moreover, their nutrient value probably diminishes rapidly with increasing complexity. The "undetermined" fraction (which must contain a good deal of purine and pyrimidine nitrogen) seems to be quite unassimilable, so that the only category left for consideration is the amide nitrogen, which is probably largely composed of asparagine and glutamine. Now asparagine does seem to be a decidedly better nutrient than ammonium phosphate (see Table I) though the reason for this is at present obscure.

Nevertheless, even without further experimental evidence, there are two reasons already apparent why amide nitrogen cannot provide the solution to the problem of yeast growth in wort: (i) its amount in wort (8 *per cent.* according to Schryver and Thomas, and 9 *per cent.* on the worts referred to above) is quite insufficient to account for the magnitude of the effect obtained. Thus the average total nitrogen content of the present worts being 0.400 *gram.* *per litre*, their average amide nitrogen content is 0.036 *gram.* *per litre*; at point A in Diagram IV, however, where the maximum difference between wort nitrogen assimilation and ammonia nitrogen assimilation occurs, this difference is 0.072 *gram.* *per litre*, or twice the amount of amide nitrogen initially present; (ii) the rate of assimilation of asparagine nitrogen carried out in the presence of ammonia (see Thorne, *ibid.*, 1945, 6) is approximately twice that of ammonia, whereas the experiments described here show that a large proportion of the wort nitrogen is no less than five times as assimilable as ammonia. Thus it may be concluded that amide nitrogen is decidedly too low both in assimilability and amount to explain the behaviour of yeast in wort. Apart from these considerations, direct estimation of the amide nitrogen (by determination of the ammonia produced during acid hydrolysis) in wort and in the corresponding beer produced from it has shown that only about 50 *per cent.* of the initial amide nitrogen is in fact assimilated by the yeast. In a particular case, a wort containing 0.943 *gram.* total nitrogen *per litre* had an amide nitrogen content of 0.081, *i.e.* 8.6 *per cent.* During the course of yeast growth the total nitrogen assimilated was 0.335 *gram.*, while the amide nitrogen assimilated was 0.039 *gram.*; this is 48 *per cent.* of the initial amide nitrogen, or 12 *per cent.* of the total assimilated nitrogen. It can be concluded that although amide nitrogen assimilation is by no means an insignificant proportion of the total it is certainly much too small to be more than a contributory factor to the high rate of assimilation of wort nitrogen.

It may be recalled that in the paper just quoted the writer demonstrated an exaggeration of nutrient differences when nutrients are used in the presence of ammonia nitrogen. Extrapolating from the data given there, a superiority of five times for wort nitrogen in the presence of ammonia implies a superiority

of about 1.7 times when they are compared separately; this agrees well with the facts (see, for example, Table I).

SECTION 4

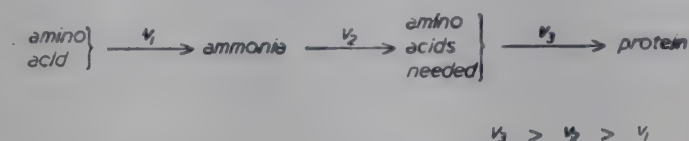
Formulation of a New Hypothesis for Yeast Growth in Wort.

The foregoing discussion should have made it clear that there is no component among the nitrogen compounds of wort which is competent, in conjunction with the known mechanisms—Ehrlich and Stickland—of nitrogen assimilation by yeast, to explain the very high assimilability of wort nitrogen. It is therefore necessary to explore the possibility that some hitherto unsuspected mechanism of assimilation functions in wort. The essential clue in this search is provided by the stimulations of yeast growth already alluded to in binary mixtures of nitrogen nutrients, other than those explicable in terms of the Stickland mechanism.

If the process is examined by which yeast utilizes a single amino acid as a source of nitrogen for the production of further proteins for its own growth it will be admitted that three conceptually distinct stages must be postulated: (i) the deamination of the amino acid with the release of ammonia; (ii) the synthesis from this ammonia of all the individual amino acids which are required for protein production; and (iii) the integration of these amino acids into the protein structures. If ammonia is used as the primary nitrogen source, stage (i) is eliminated and under these conditions, as already noted, growth is faster; it must therefore be admitted that the overall velocity of the reactions involved in this stage is slower than that of either stage (ii) or stage (iii). The hypothesis which is now proposed is that if stage (ii) as well as stage (i) is eliminated, that is to say, if yeast is supplied with an *adequate mixture* of amino acids, no deamination will be necessary at all since the amino acids will be present ready for direct building up into protein. If it is assumed that the overall velocity of the reactions in stage (iii) is greater than that of stage (ii), then the unexpectedly high assimilability of wort nitrogen will be accounted for. For the amino acids present in wort, derived from the degradation of barley proteins, are likely to be entirely adequate qualitatively for the production of yeast proteins; if they do not

need to be deaminated and resynthesized but can be utilized intact then the two slower stages in the postulated reaction chain will be by-passed and nitrogen assimilation can proceed at the maximal rate. The simple scheme, embodying this new hypothesis is illustrated in Diagram V, where v_1 , v_2 and v_3 represent the overall reaction velocities of the three stages.

DIAGRAM V



Three stages in the utilization of an amino acid by yeast

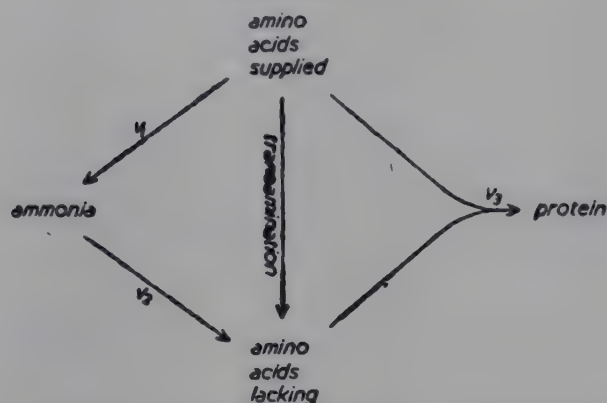
The main purpose of the subsequent sections of this paper will be to examine the consequences of this working hypothesis, which may now be formally stated as follows:—

When yeast is supplied with a diverse mixture of amino acids they will tend to be assimilated intact and at a higher rate than would be possible if they had to undergo deamination.

It is a reasonable inference from this hypothesis that a mixture of amino acids not quite adequate for the synthesis of yeast protein, *i.e.*, a mixture lacking a few necessary amino acids would nevertheless still have superior nutrient properties to individual amino acids since the synthesis of the lacking amino acids would involve only a small amount of deamination of the remainder. It may therefore be legitimate to regard the small stimulations of yeast growth encountered in binary amino acid mixtures as a relic of the much bigger stimulations in complex mixtures. If this is so, it may be expected that amino acid mixtures will normally be better nutrients than single amino acids, the extent of the superiority of such mixtures depending primarily on the number of their constituents; some evidence in favour of this view will be presented below. In place of the simple scheme of Diagram V that of Diagram VI may therefore be preferred as probably closer to reality. It expresses the view that the amino acids supplied to yeast do not need to be deaminated except to the extent necessary to synthesize the amino acids

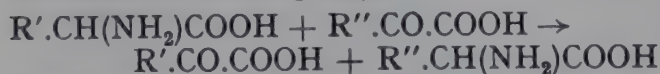
required but not supplied. Consequently, although the rate of deamination by the Ehrlich and Stickland mechanisms imposes a limitation upon the rate of yeast growth when many amino acids have to be synthesized from the resulting ammonia, the deamination rate may be quite adequate to allow of rapid yeast growth when the ammonia is required for the synthesis of only a few amino acids. It is also possible that if yeast contains several deaminases with differing specificities a more rapid ammonia supply may be produced from a mixture of amino acids than from a single one.

DIAGRAM VI



Proposed scheme for the utilization of mixed amino acids by yeast

Diagram VI indicates also that the deamination mechanisms for the synthesis of missing amino acids may be supplemented by transamination mechanisms. Transamination is a process whereby an α -amino acid and an α -keto acid suffer an interchange of amino and keto groups:



Although it is suspected that this process may be a general one the evidence is scanty. Some important work by Roine (*On the formation of primary amino acids in the protein synthesis in yeast*; Doctoral dissertation, Helsinki, 1947) has demonstrated, however, that in *Torulopsis* yeast glutamic acid, aspartic acid, alanine, valine, leucine and isoleucine may participate in transamination reactions.

SECTION 5

The Growth of Yeast in Synthetic Media Containing Mixed Amino Acids.

It is now necessary to examine the consequences of the proposed hypothesis of intact

assimilation of amino acids in order to submit it to thorough experimental test.

If the superior growth of yeast in wort is really due to the operation of the mechanism suggested then it is clear that a similar effect should be obtained with a synthetic medium containing a complex mixture of amino acids as the nitrogen source. The experiments designed to test this point were carried out in essentially the same way as the experiments with wort already described, that is to say, in synthetic media containing several amino acids as nitrogen source together with ammonium phosphate as a comparison source, simultaneous estimations of ammonia and total nitrogen uptake (giving amino nitrogen uptake by difference) being made during the course of yeast growth. The composition of the basal medium was:—

- | | |
|--|---|
| 1,000 ml. standard mineral salt solution | } |
| 60 grms. sucrose | |
| 0.01 grm. ferrous sulphate | |
| 0.02 grm. inositol | |
| 2 microgrms. biotin | |
| 2 mgrms. pantothenic acid | |
| 0.1 mgrm. aneurin | |
| 0.1 mgrm. pyridoxin | |
| 3.5 ml. lactic acid | |
| 12 ml. 50 per cent. potassium lactate | |
| 0.3 grm. nitrogen as amino acids | } |
| 0.2 grm. nitrogen as ammonium phosphate | |

The results obtained with seven such nutrient media are given in Table VI. These media (I to VII) were not quite identical with respect to their amino acid composition, but since no special significance appears to attach to the differences between them, only their average composition will be quoted here; this was as follows:—

Glycine	..	0.023	} Grm. nitrogen per litre contributed by each amino acid.
Alanine	..	0.044	
Leucine	..	0.028	
isoLeucine	..	0.012	
Glutamic acid		0.068	
Aspartic acid		0.023	
Arginine	..	0.013	
Phenylalanine		0.006	
Tyrosine	..	0.022	
Histidine	..	0.043	
Tryptophane	..	0.022	

As with Table V, the last column of Table VI shows the average extents of amino nitrogen consumption for successive levels of ammonia nitrogen consumption and incorporates

TABLE VI

ASSIMILATION OF NON-AMMONIA AND AMMONIA NITROGEN FROM SEVEN AMINO ACID MIXTURES WITH ADDED AMMONIUM PHOSPHATE

(All quantities expressed in grms. per litre)

	I.	II.	III.	IV.	V.	VI.	VII.	Means.	Means corrected for concentration.
(a)*	0.325	0.380	0.240	0.270	0.280	0.333	0.312	0.306	
(b)†	0.141	0.138	0.200	0.221	0.243	0.246	0.230	0.203	
Ammonia nitrogen uptake.	Non-ammonia nitrogen uptake.								
0.010	0.028	0.017	0.025	0.018	0.021	0.013	0.038	0.023	0.022
0.020	0.047	0.034	0.037	0.022	0.024	0.025	0.064	0.036	0.034
0.030	0.053	0.056	0.038	0.025	0.033	0.033	0.082	0.046	0.044
0.040	0.084	0.082	0.049	0.030	0.065	0.046	0.096	0.065	0.062
0.050	0.085	0.096	0.067	0.045	0.072	0.072	0.120	0.080	0.076
0.060	0.103	0.121	0.080	0.066	0.080	0.084	0.134	0.095	0.091
0.070	0.133	0.130	0.089	0.057	0.094	0.095	0.148	0.107	0.102
0.080	0.145	0.137	0.093	0.070	0.099	0.105	0.162	0.116	0.111
0.090	0.157	0.160	0.097	0.083	0.109	0.115	0.174	0.128	0.122
0.100	0.171	0.184	0.110	0.096	0.122	0.124	0.185	0.142	0.135
standard deviation									± 0.009

*(a) = Initial non-ammonia nitrogen.

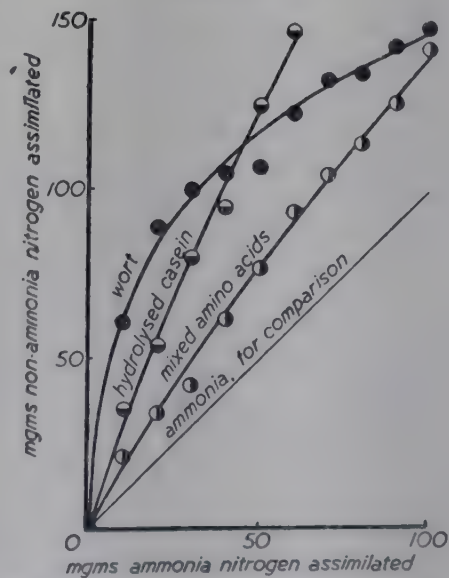
†(b) = Initial ammonia nitrogen.

a small correction to allow for the unequal initial concentrations of these two forms of nitrogen in the nutrient media. These values are shown graphically in Diagram VII which also, for comparison, shows the curve of wort assimilability already discussed. It is at once evident that although the mixture of 11 amino acids does not give such rapid assimilation of amino nitrogen as is found in wort, it is nevertheless much better than ammonia. The initial rate of amino nitrogen assimilation is greater than the final rate, but the difference is less marked than in the case of wort. The overall rate of assimilation of amino nitrogen is 1.5 times that of ammonia, which corresponds to just double the average nutrient value of the constituent single amino acids. The evidence of these experiments is therefore in favour of the hypothesis.

It was natural to suppose that the disparity between the rate of uptake of wort nitrogen and of the nitrogen of the foregoing amino acid mixtures is due to the wort containing a much more diverse mixture of amino acids. This point could readily be tested, using the same technique as before, by growing yeast in a synthetic medium containing casein hydrolysate as the source of nitrogen together, of course, with ammonium phosphate. Casein

hydrolysate contains practically all the known naturally occurring amino acids except tryptophane, which is destroyed by the acid

DIAGRAM VII



Assimilabilities of artificial amino acid mixtures, hydrolysed casein and wort

hydrolysis (and for the same reason it is devoid of amide nitrogen); consequently, it would be expected to show a higher assimilability than a mixture of only 11 amino acids.

The results of a set of six experiments which were carried out exactly as before, merely replacing the artificial mixture of amino acids by casein hydrolysate (free from growth factors), are given in Table VII and shown graphically by the appropriate curve in Diagram VII. Here there is not, in comparison with wort, such a strongly marked decline in the rate of assimilation during the course of yeast growth.

points. Multiplying these figures by a factor of 1.5 gives the approximate rates of assimilation compared with the average single amino acids. It may be concluded that the figures for casein hydrolysate showing as they do, not only a higher rate of assimilation than ammonia, but also a higher rate than that given by the simpler artificial amino acid mixtures again support the hypothesis. The discrepancy remaining between wort and

TABLE VII
ASSIMILATION OF NON-AMMONIA AND AMMONIA NITROGEN FROM SIX CASEIN HYDROLYSATES WITH
ADDED AMMONIUM PHOSPHATE
(All quantities expressed in grms. per litre)

	I.	II.	III.	IV.	V.	VI.	Means.	Means corrected for concentration.
(a)*	0.131	0.253	0.234	0.277	0.169	0.171	0.206	
(b)†	0.231	0.251	0.252	0.253	0.195	0.190	0.229	
Ammonia nitrogen uptake.	Non-ammonia nitrogen uptake.							
0.010	0.023	0.045	0.014	0.072	0.025	0.028	0.035	0.035
0.020	0.032	0.063	0.048	0.099	0.049	0.039	0.055	0.056
0.030	0.054	0.093	0.071	0.127	0.084	0.059	0.081	0.082
0.040	0.069	0.103	0.092	0.139	0.093	0.080	0.096	0.097
0.050	0.086	0.147	0.133	0.185	0.101	0.103	0.126	0.127
0.060	0.095	0.175	0.174	0.200	0.129	0.118	0.149	0.151
0.070	0.101	0.193	0.173	0.215	0.138	0.132	0.159	0.161
0.080	0.107	0.212	0.183	0.231	0.147	0.147	0.171	0.173
standard deviation								± 0.014

(a) = Initial non-ammonia nitrogen. †(b) = Initial ammonia nitrogen.

TABLE VIII
RATES OF ASSIMILATION OF VARIOUS SOURCES OF
NITROGEN COMPARED WITH AMMONIA AS UNITY

	Data in	Initial rate.	Overall rate.
Wort (diluted) ..	Table V	5.1	2.1
„ (undiluted) ..	„ XII	5.2	3.9
Casein hydrolysate ..	„ VII	3.0	2.4
Eleven amino acids ..	„ VI	1.9	1.5

Moreover the rate is, as was anticipated, much higher than that of the previous mixture of amino acids. It may be useful to summarize the results now obtained for rates of assimilation compared with ammonia; this is done in Table VIII. Initial rates are obtained by averaging the values given by the first two points of the curves, while overall rates are obtained by using all the

casein hydrolysate is of some interest. The fact that the overall rate in casein hydrolysate is higher than that in wort probably reflects some shortage of amino acids in the latter. The superior initial rate in wort is probably due to the presence of asparagine.

SECTION 6
*The Utilization of Carbohydrate under Different
Conditions of Nitrogen Nutrition.*

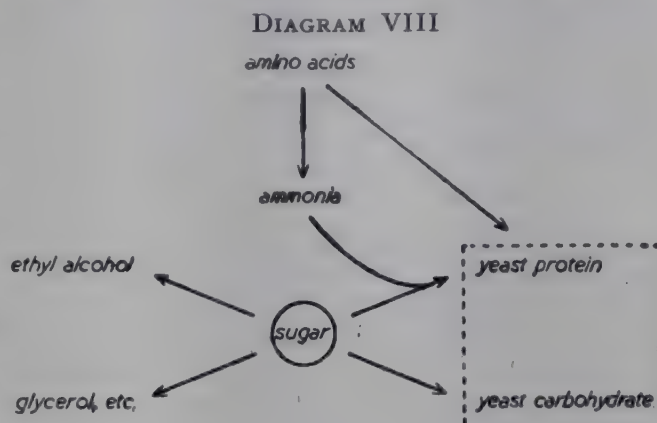
If the hypothesis of the intact assimilation of amino acids from complex mixtures is true then it follows that under these conditions the amino acids will be supplying not only nitrogen but also carbon for the construction of yeast proteins. The considerations involved are set out in Diagram VIII, which shows that there are four main pathways by which sugar is utilized by growing yeast: (i) the formation of ethyl

alcohol by fermentation; (ii) the formation of by-products such as glycerol, succinic acid and acetic acid; (iii) the formation of the carbohydrate and fatty constituents of the yeast: these amount to about 40 *per cent.* of the yeast dry weight (or 80 *per cent.* of the non-protein portion); and (iv) the formation, in conjunction with ammonia, of yeast proteins which amount to some 50 *per cent.* of the yeast dry weight. In addition, a certain amount of sugar may undergo complete oxidation to carbon dioxide and water. If the nitrogen source is actually ammonia, or effectively ammonia as in the case of a single amino acid which has to undergo deamination, then the carbon required for protein building must come from the sugar if the latter is the only carbon compound in the medium. If, on the other hand, the nitrogen source is a mixture of amino acids (*e.g.*, casein hydrolysate) the sugar will be spared to the extent that the amino acids are being built directly into proteins.

These considerations thus form the basis of another method of testing the hypothesis. If a nutrient medium containing an accurately measured quantity of glucose is fermented until the glucose is completely exhausted the amount fermented may be deduced by estimation of the ethyl alcohol production. Determination of the nitrogen assimilated (multiplied by the conventional factor of 6.25) will give an estimate of the amount of yeast proteins formed; the yeast dry weight less this protein figure multiplied by 0.8 will give approximately the amount of glucose used up to form yeast carbohydrate. Of the glucose still unaccounted for some will have been consumed in the formation of glycerol and other by-products: the amount involved in this way is not readily determined but normally amounts to some 4 *per cent.* of the sugar initially present. Another part of the sugar remaining unaccounted for will have been oxidized and an upper limit to this can be put by comparing the production of carbon dioxide with that of ethyl alcohol. In the experiments to be described the average production of carbon dioxide exceeded that of alcohol by an amount equivalent to the complete oxidation of 1 *per cent.* of the initial glucose; this may mean, of course, incomplete oxidation of a larger amount of sugar.

Essentially the experiments consisted in making up five sets of nutrient media with an accurately measured concentration of

glucose, and with ammonium phosphate, leucine, glutamic acid, arginine and casein hydrolysate respectively as the sources of nitrogen. (The usual lactic acid-potassium lactate buffer was excluded from the media as it would introduce a possible alternative carbon source to the yeast.) The media were fermented with yeast 6479 until carbon dioxide evolution ceased, the exhaustion of the glucose being verified by testing with Fehling's solution. The initial glucose concentration was at a fairly low level, 25 grms.



Utilization of sugar by yeast

per litre, in order that no difficulty should be experienced in completely metabolizing it. The ethyl alcohol in the fermented media was determined by the usual method of distillation and from it the amount of glucose fermented was calculated. (The loss of alcohol by evaporation during the fermentation was determined by separate controls and found to be negligible.) Determinations of yeast dry weight and nitrogen assimilation were also made. It was to be anticipated that with casein hydrolysate as the nitrogen source more ethyl alcohol would be produced as the amino acids would be built into protein without deamination, thus sparing an equivalent amount of glucose.

The mean results of six such comparisons are recorded in Table IX. The derivation of the figures in the first five columns of this table has already been dealt with. The sixth column—glucose consumed in other ways—is obtained by subtracting the glucose fermented and the glucose equivalent to the yeast carbohydrate from the initial glucose. It therefore includes glucose which has been consumed by oxidation and in the formation of by-products as well as in the formation of yeast proteins. It will be seen that when casein hydrolysate

TABLE IX
FATE OF GLUCOSE DURING FERMENTATION WITH DIFFERENT NITROGEN SOURCES
(Mean result of six experiments. All quantities expressed in grms. *per* litre)

	Dry yeast formed.	Nitrogen assimilated.	Initial glucose.	Glucose fermented.	Glucose equivalent to yeast carbohydrate.	Glucose consumed in other ways.	Glucose-sparing effect.	Glucose equivalent to yeast protein.
Ammonium phosphate	2.15	0.177	25.00	18.38	0.84	5.78	—	—
Leucine	1.65	0.119	25.00	20.56	0.72	3.72	—	—
Glutamic acid	2.02	0.157	24.64	19.46	0.83	4.35	—	—
Arginine	2.21	0.202	25.00	19.57	0.76	4.67	—	—
Casein hydrolysate ..	2.40	0.215	25.00	21.18	0.85	2.97	1.28	1.85
Standard deviation ..	± 0.06	± 0.004	—	± 0.18	± 0.03	± 0.19	± 0.16	± 0.03

is the nitrogen source the glucose remaining unaccounted for is less than that with either of the single amino acids—this is, in fact, the glucose sparing effect which was sought: its magnitude is taken as the difference between casein hydrolysate and the mean of the three separate amino acids and is given in the seventh column of the table. Although this difference, 1.28, is a relatively small one and is given by the difference between two larger figures which are themselves subject to experimental error, it should be emphasized that a statistical test (*t*-test) shows that the difference in this respect between casein hydrolysate and the separate amino acids is overwhelmingly significant, there being far less than 1 chance in 100 that such a difference could arise fortuitously. The protein in the yeast can be calculated from the nitrogen assimilation figure and from this (assuming an average content of 55 *per cent.* of carbon in protein) the glucose required to produce it if ammonia were the nitrogen source can be calculated; this figure, 1.85, is shown in the last column and may be regarded as the maximum possible sugar sparing effect. The actual sugar sparing effect is 69 (± 9) *per cent.* of this. The evidence of these experiments is therefore entirely in favour of the hypothesis of intact assimilation of amino acids and suggests that under the conditions employed two-thirds of the amino acids of casein hydrolysate are assimilated in this way.

It will be noticed that the amount of glucose consumed in ways other than fermentation and formation of yeast carbohydrate is greater with ammonium phosphate

as the nitrogen source than with single amino acids. The cause of this is obscure, but possible contributory reasons are: (i) the actual yield of yeast and assimilation of nitrogen are greater with ammonium phosphate; (ii) the excess of carbon dioxide production over ethyl alcohol production indicates that more sugar undergoes oxidation when ammonia is the nitrogen source; (iii) the amino acids may lead to some conservation of glucose by participation in transamination reactions; and (iv) there may be an appreciable amount of direct assimilation of the amino acids.

SECTION 7

Pyridoxin Requirements of Yeast with Different Nitrogen Sources.

In some recent experiments the writer (this *Journ.*, 1949, 18) found reason to suspect that the requirement of yeast for pyridoxin (vitamin B₆) was somewhat greater when single amino acids were the source of nitrogen than when ammonia was used. This seemed to suggest that pyridoxin is involved in the mechanism of deamination of amino acids. Pyridoxin (or rather, the closely-related pyridoxal phosphate) is already known to be the coenzyme of bacterial amino acid decarboxylases (Baddiley and Gale, *Nature*, 1945, 155, 727) and also of bacterial transaminase (Lichstein, Gunsalus and Umbreit, *J. biol. Chem.*, 1945, 161, 311). If this suggested third rôle for pyridoxin is true, then in a medium already containing ammonia as a source of nitrogen, its presence

TABLE X

THE EFFECT OF PYRIDOXIN DEFICIENCY ON THE GROWTH OF *S. CARLSBERGENSIS* WITH DIFFERENT NITROGEN SOURCES

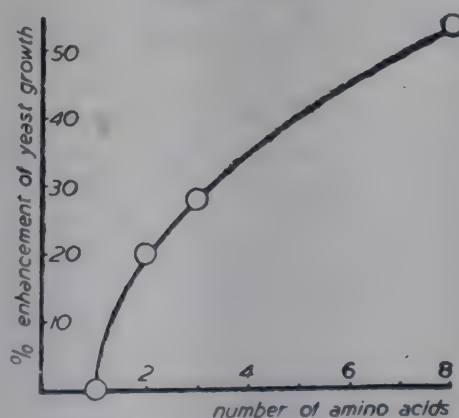
Nitrogen source.	Pyridoxin concentration γ /l.	Yeast growth grms./l.	Per cent. depression.	Weighted means.
Ammonium phosphate ..	100	18.0	—	—
Leucine		11.2	—	—
Glutamic acid		21.0	—	—
Arginine		18.8	—	—
Casein hydrolysate ..		23.1	—	—
Ammonium phosphate ..	10	15.6	13	13
Leucine		7.1	37	27
Glutamic acid		17.9	15	
Arginine		12.4	34	
Casein hydrolysate ..		18.8	19	19
Ammonium phosphate ..	1	11.8	34	34
Leucine		4.7	58	52
Glutamic acid		11.9	43	
Arginine		7.7	59	
Casein hydrolysate ..		17.6	24	24
Ammonium phosphate ..	0	11.3	37	37
Leucine		4.7	58	56
Glutamic acid		10.5	50	
Arginine		7.2	62	
Casein hydrolysate ..		12.4	46	46
Standard deviation		± 1.8		

should be more dispensable. Moreover, in a medium whose source of nitrogen is an adequate mixture of amino acids these, according to the hypothesis of this paper, should be assimilated without deamination and the requirement for pyridoxin should be smaller, resembling that of ammonia rather than single amino acids. This then constitutes the basis of a third independent method of testing the validity of the hypothesis.

The experiments were conducted with a yeast, *S. carlsbergensis* (Fleischmann, No. 4228), which is particularly sensitive to pyridoxin deficiency. The basal medium already specified was used, with any one of five different nitrogen sources: ammonium phosphate, leucine, glutamic acid, arginine and casein hydrolysate. The effect on yeast growth of gradual diminution of the pyridoxin supply was measured by using each of the nitrogen sources in four media containing respectively 100, 10, 1 and 0 microgrms. of pyridoxin *per* litre. The experimental results showing yeast growth in the various media after three days growth at 21° C. with constant shaking, are given in Table X.

These results are the means of two sets of experiments, each done in triplicate, so that each yeast growth value is the mean of six observations. The column of the table headed percentage depression gives

DIAGRAM IX



Relation between nutrient value and complexity of amino acid mixtures

the amount, expressed as a percentage of the growth with 100 microgrms. of pyridoxin *per* litre, by which the growth with the same nitrogen source but a reduced pyridoxin concentration falls short of that with the

maximum pyridoxin concentration. Thus, for ammonium phosphate with 10 microgrms. of pyridoxin *per* litre the percentage depression is $100 \times (18.0 - 15.6)/18.0 = 13$.

It is quite clear that the single amino acids are more sensitive to pyridoxin deficiency than either ammonia or casein hydrolysate; this is summarized in the weighted means for the amino acids given in the last column of the table. A statistical test shows that the differences between the figures for ammonia and individual amino acids are highly significant, the chance of getting such results fortuitously being less than 1 in 100. On the other hand, the differences between the results for ammonia and casein hydrolysate have no statistical significance at all. In other words, casein hydrolysate—as far as pyridoxin requirement is concerned—behaves similarly to ammonium phosphate and in marked contrast to single amino acids. The hypothesis of intact assimilation therefore receives further support. It will be noticed that there is a consistent difference—which is statistically significant—between glutamic acid and the other two amino acids, and it may be that the amino acids in general are not quite uniform in their pyridoxin requirements; such small differences might be due to varying amounts of intact assimilation supplementing the deamination mechanism. As in the previous experiment the particular three amino acids used here were selected as belonging to three distinct types (neutral, acidic and basic) in order to make as representative a small sample as possible.

SECTION 8

Other Evidence for the Hypothesis of Intact Assimilation of Amino Acids.

The scheme of nitrogen assimilation from amino acids expressed in Diagram VI implies that when yeast is furnished with a mixture of all the amino acids necessary for the construction of its proteins they are assimilated without deamination, yeast growth proceeding in consequence at the maximum possible rate. If, however, a necessary amino acid is lacking some of those supplied will have to be deaminated in order that the missing ones may be synthesized from ammonia; consequently, yeast growth will be somewhat retarded. As further amino acids are eliminated from the mixture, yeast

growth will become progressively slower until the limit is reached when only a single amino acid is supplied. Looked at from the opposite point of view, the average nutrient value of binary mixtures should show an enhancement over that of single amino acids, ternary mixtures should be better than binary, and so on. The present writer has published data for yeast growth in binary mixtures (this *Journ.*, 1944, 186; 1946, 5, 15) and in ternary mixtures (*ibid.*, 1946, 5); experiments (unpublished) have also been made on mixtures containing eight amino acids. The average enhancements (compared with the corresponding single amino acids) are 20 *per cent.* for binary mixtures, 28 *per cent.* for ternary mixtures and 54 *per cent.* for mixtures of eight nutrients. These values are illustrated graphically in Diagram IX and show a progressive improvement in yeast growth with increasing complexity of the nitrogen source, just as the hypothesis of intact assimilation of amino acids requires.

It now becomes possible also to interpret the enhancements obtained in mixtures one of whose constituents is ammonia (see Table III). Since ammonia can be used directly for the synthesis of the necessary amino acids, the rate of deamination of the amino acid supplied will not be a limiting factor on yeast growth. On the other hand, the one amino acid present in the nutrient mixture can be assimilated intact to the extent that it is needed for protein formation; since yeast will be spared the necessity of synthesizing it a small enhancement of growth should normally be observable.

Another interesting confirmation of the hypothesis of intact assimilation has been very kindly made known to the writer by Dr. E. C. Barton-Wright (private communication). Barton-Wright, using the methods of microbiological assay, has followed the assimilation of numerous individual amino acids during yeast growth in brewery wort. Among these was lysine, which is conspicuous among amino acids in being completely resistant to deamination when used as the sole source of nitrogen (see Table I; see also Nielsen, *Compt. rend. Trav. Lab. Carlsberg*, 1936, 21, 395); in other words, it is unable alone to support yeast growth. From wort, however, where it is present alongside many other amino acids it is assimilated by yeast with great rapidity. Averaged figures from

the data supplied by Barton-Wright are given in Table XI from which it may be

TABLE XI

ASSIMILATION OF LYSINE FROM WORT

(E. C. Barton-Wright, private communication)

Hours after inoculation.	Grms. of lysine nitrogen <i>per</i> litre.
0	0.030
10	0.011
20	0.005
40	0.005
80	0.005

inferred that the assimilation of lysine has reached its limit within about 15 hours from the commencement of yeast growth. Similar results have been obtained by Barton-Wright with histidine, an amino acid which falls little short of lysine in its resistance to deamination. Although the possibility of the participation of these amino acids in Stickland interactions, under the conditions obtaining in wort, is not excluded it does not seem possible that their extremely rapid assimilation from wort could be accounted for in this way. The assimilation of 0.030 grm. of lysine nitrogen from 1 litre of wort in 15 hours is considerably faster than the rate of assimilation of ammonia nitrogen when it is mixed with wort as in the experiments described above. Consequently, it seems legitimate to regard these data of Barton-Wright as contributing further support to the hypothesis of intact assimilation.

SECTION 9

Review of the Experimental Evidence.

The very much greater assimilability of wort nitrogen than that of ammonia, which was unpredictable from the Ehrlich and Stickland mechanisms and the known behaviour of individual amino acids, led to the proposal of the hypothesis that the amino acids when present in a sufficiently complex mixture are assimilated directly without deamination. Various deductions were made from this hypothesis and submitted to experimental test:—

- (i) The assimilation of nitrogen from artificial mixtures of several amino acids should be more rapid than that of ammonia. This was found to be true.

- (ii) A richer mixture of amino acids such as that present in casein hydrolysate should give a still greater improvement. This also was found to be true, the rate of assimilation of nitrogen from casein hydrolysate being not much inferior to that from wort.

- (iii) The assimilation of intact amino acids should result in more carbohydrate being available for fermentation. This was found to be true. The observed sugar-sparing effect was about two-thirds of the maximum possible effect.

- (iv) If, as there is reason to think, pyridoxin is involved in the deamination of amino acids, complex amino acid mixtures as well as ammonia should be more able to dispense with it than single amino acids. This was found to be true.

- (v) Starting with a single amino acid in a nutrient medium, the addition of other amino acids one by one should result in a progressive improvement in the nutrient medium. This also has been found to be true.

- (vi) The addition of an amino acid to ammonia should give some improvement of yeast growth. This has been found to be generally true.

- (vii) Essential amino acids which yeast is unable to deaminate should be assimilated intact from complex mixtures. The observations of Barton-Wright imply that this is true.

Thus, of the seven types of prediction from the hypothesis which it has been possible to submit to experimental examination, all have given evidence unequivocally in its favour. No one line of evidence would be claimed as conclusive, but the agreement of all seven is, in the writer's opinion, sufficient to render the hypothesis worthy of serious attention.

Two further possibilities which have not been examined experimentally are:—

- (viii) The production of fusel oil (the deamination residues of amino acids) during yeast growth in wort should be significantly lower than that to be expected on the Ehrlich hypothesis. No experimental test of this has been made because the residues from only

eight amino acids are known with certainty. Even if all the residues were known their isolation and estimation would be subject to a very large experimental error; the low recoveries which are normally obtained in this kind of work would lend only a fictitious support to the hypothesis.

- (ix) If yeast were supplied with a mixture of amino acids each containing an isotopically or radioactively labelled carbon atom, these labelled atoms should be transferred largely to the yeast protein where their amount could be estimated. This should constitute the most conclusive test of the hypothesis and should also offer a means of measuring its extent, but the means to carry it out are not available to the writer.

SECTION 10

A Unified View of Nitrogen Assimilation from Wort.

Enough information is now available to attempt to draw a coherent picture of nitrogen assimilation by yeast from wort. Wort presents yeast with a diverse mixture of amino acids which on account of their rapid disappearance must be taken up largely without deamination during the earlier stage of yeast growth. At the end of this phase of rapid assimilation a second phase of markedly slower assimilation sets in. There are two possible reasons which may account for this:—

- (i) The supply of free amino acids may be practically exhausted during the period of rapid assimilation; further amino acids for yeast growth could then only arise by hydrolysis of the simple polypeptides present in wort, but as shown by Damlé and Thorne (*loc. cit.*) since the rate of yeast growth in dipeptides tends to be slower than in the corresponding binary mixtures of amino acids this must constitute a severe check on the rate of nitrogen assimilation.
- (ii) Unless the amino acid composition of wort is nearly identical with that of yeast proteins—which is improbable—the yeast, as it selects out the amino

acids it requires, will leave unassimilated a progressively more inadequate mixture; in order to restore the increasing deficiency of necessary amino acids, those remaining unassimilated will have to be attacked by the Ehrlich and Stickland mechanisms which again will severely retard the rate of nitrogen assimilation.

There are good reasons to suppose that both of these processes are operative. The mere fact that fusel oil is a by-product of fermentation is enough to prove that the Ehrlich mechanism is involved. Moreover, since a significant amount of nitrogen is required by yeast for the production of nucleic acids whose nitrogen exists in the form of purine and pyrimidine bases, deamination of amino acids must presumably occur to provide ammonia for the synthesis of these. The fact that, although the initial amino nitrogen content of wort is much less than its content of assimilable nitrogen, amino nitrogen still remains in considerable amounts in the fermented wort (Windisch, Kolbach and Hennecke, *Woch. Brau.*, 1928, 45, 389 *et seq.*; Nielsen and Lund, *Compt. rend. Trav. Lab. Carlsberg*, 1936, 21, 239) suggests that peptides are being hydrolysed to amino acids which are then assimilated as required.

The question of the assimilation of amide nitrogen, which amounts to 8 to 9 *per cent.* of the total nitrogen of wort (though some of this is probably bound up in peptides), also demands some attention. In view of the high assimilability of asparagine compared with ammonia (see Table I) it seems likely that a considerable proportion of this amide nitrogen will be taken up by yeast during its growth in wort. Experiments quoted in Section 3 have shown that in fact some 50 *per cent.* of it is utilized. This may well be the reason for the more rapid assimilation of nitrogen from wort than from casein hydrolysate. Support for this conclusion was obtained from an experiment in which the rate of assimilation of nitrogen from a mixture of amino acids was compared with the rate from the same mixture plus an addition of asparagine: the rate in the second case was augmented by an amount which just corresponded with a rapid assimilation of the asparagine. The reason for the high nutrient value of asparagine is unknown, but it is likely that, together with aspartic acid, it

participates in some transamination system. In this connection it is interesting to note that Roine (*Suomen Kemistilehti*, B, XIX, 1946, 73) has found that when yeast is grown with ammonia as the source of nitrogen the first soluble nitrogen compounds to appear within the yeast cell are glutamic and aspartic acids together with their amides. This finding is in consonance with the rather high nutrient value of these substances in comparison with other amino acids.

At this point it is desirable to mention some experiments carried out on the lines of those in Section 3, but using undiluted worts instead of the diluted worts employed there. It is to be expected that as the nitrogen content of a wort increases so will the proportion of amino acids which are susceptible to assimilation by direct absorption; consequently, the very high assimilation rate should endure for a longer period than was the case with diluted worts. These expectations are realized in the results given in Table XII, which are mean figures derived from experiments carried out on

TABLE XII

ASSIMILATION OF NON-AMMONIA AND AMMONIA NITROGEN FROM UNDILUTED WORTS CONTAINING ADDED AMMONIUM PHOSPHATE

(Mean result of seven experiments. All quantities in grms. *per* litre)

Ammonia nitrogen uptake.	Non-ammonia nitrogen uptake.
(b)† 0.407	(a)* 1.058
0.010	0.051
0.020	0.104
0.030	0.141
0.040	0.163
0.050	0.196
0.060	0.231
0.070	0.246
0.080	0.264
Standard deviation $\pm$ 0.023	

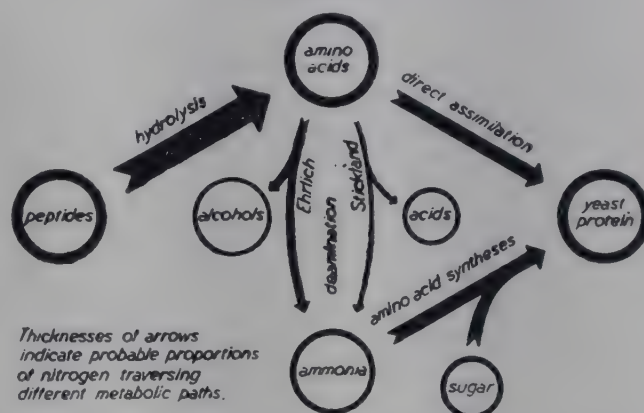
* (a) = Initial non-ammonia nitrogen.

† (b) = Initial ammonia nitrogen.

seven worts of sp.gr. 1.045 made from malts of varying nitrogen content. Two conclusions are to be drawn from these figures: (i) the initial rate of wort nitrogen assimilation is approximately five times that of ammonia nitrogen, agreeing closely in this respect with the results of Table V for diluted worts;

(ii) the subsequent decline in the rate of wort nitrogen assimilation is very much less marked than in the case of diluted worts. The latter, in Table V, had a mean nitrogen content of 0.400 gm. *per* litre, of which 0.090 gm. appeared to be assimilated by direct absorption. The undiluted worts of Table XII have a mean nitrogen content of 1.058 gm. *per* litre, which would imply a content of approximately 0.24 gm. nitrogen assimilable by direct absorption. This is so near to the total assimilation that it is not surprising that only a slight falling off is observable in the rate of assimilation. The use of undiluted wort thus fully confirms the previous estimate of wort nitrogen initial assimilability but is ill adapted to determining what proportion of wort nitrogen possesses this very high assimilability.

DIAGRAM X



The assimilation mechanisms operative in wort

As far as the principal source of yeast nitrogen—amino acids—is concerned, it is worth attempting to estimate the relative importance of the three mechanisms of assimilation: intact absorption, Ehrlich deamination and Stickland deamination. The data recorded in Diagram IV suggest that of a total of 150 mgrms. of nitrogen 90 are assimilated by the intact absorption of amino acids. Actually the nitrogen assimilation continues beyond the point marked B in this graph and tails off at a level of approximately 180 mgrms. The assimilation of intact amino acids therefore accounts for about 50 *per cent.* of the total nitrogen uptake. The remaining 50 *per cent.* of the nitrogen must undergo deamination. Only the most shadowy estimate of the importance of the Stickland mechanism relative to that of Ehrlich can be made. The calculation in

Section 2 shows that the latter must be far the more important of the two; it is probable that at least 40 *per cent.* of the amino nitrogen is deaminated by the Ehrlich mechanism and less than 10 *per cent.* by that of Stickland. The estimate of 50 *per cent.* for the amount of nitrogen assimilated intact may be compared with the estimate made in Section 6, namely, 70 *per cent.*, for the amino nitrogen assimilated intact from casein hydrolysate. It is also to be remembered that the estimate of 50 *per cent.* is based on experiments with diluted worts; with stronger worts it is much more difficult to make an estimate at all but it seems a reasonable inference that, from such worts, considerably more than half the total assimilation would be accounted for by intact absorption. Diagram X, which is self-explanatory, gives a concise summary of the mechanisms of nitrogen assimilation as they are held to occur in wort.

The Ehrlich mechanism has for so long been regarded as an adequate theoretical foundation for the study of nitrogen assimilation by yeast that the hypothesis of intact

assimilation of amino acids may appear somewhat novel. This is not really so, however, for it is now well known that many micro-organisms are nutritionally exacting towards amino acids; that is to say, on account of their loss of certain synthetic abilities they must be supplied with particular amino acids in order to grow at all. This clearly implies that such essential amino acids are assimilated intact. What is more remarkable as far as yeast is concerned is that, despite centuries of culturing in breweries in a rich medium containing presumably all the necessary amino acids, it still retains the ability to synthesize every one of its amino acids as is shown by its growth in media whose sole source of nitrogen is ammonia.

The writer wishes to express his thanks to Mrs. Doreen L. Bradley, Miss Myra G. Hulse and Miss Muriel Southall, who have assisted with this work.

Institute of Brewing Research Laboratories,
University of Birmingham.

21st May, 1948.

RETIREMENT OF MR. JULIAN L. BAKER

It is with great regret that there has to be announced the retirement from the editorship of this *Journal* of Mr. Julian L. Baker, F.C.G.I., F.R.I.C., in consequence of ill-health. Mr. Baker succeeded Professor A. R. Ling as editor in July, 1920, and during his 29 years of office the *Journal* has achieved an eminence the influence of which continues to expand. It must be of considerable satisfaction to Mr. Baker that, under his direction, the *Journal* has attracted research contributions of great merit both from at home and from abroad, as well as providing an invaluable record of the proceedings of the Institute and of its various Sections; Mr. Baker's own original contributions to the *Journal* may also be recalled. Besides his

editorial work, Mr. Baker has made a wide contribution to the work of the Institute in other directions; thus, he has served on Council since 1907, was Hon. Secretary during the period 1909-18, and has been a Vice-President since 1918—a record of service it would be difficult to equal. He was awarded the Horace Brown Medal in 1948. All members of the Institute, and all other readers of the *Journal*, will wish Mr. Baker such a return of health and strength as will allow him to enjoy his retirement to the full.

The Council of the Institute has appointed Dr. I. A. Preece, M.Sc., F.R.I.C., F.R.S.E., to succeed Mr. Baker as editor for the immediate future.

MEETING OF THE SCOTTISH SECTION HELD AT THE NORTH BRITISH
HOTEL, EDINBURGH, ON TUESDAY, 18TH JANUARY, 1949

Mr. L. FLETCHER in the Chair

The following paper was read and discussed:—

THE SELECTION OF MALTING BARLEY

By A. M. SLIGHT

The importance of an early return to the growing of barley varieties chosen for malting quality rather than for yield or other non-malting properties is emphasized. Modern harvesting methods are discussed in relation to their influence on malting characters. The assessment of malting qualities in a sample is described, attention being paid to ripeness, shape of corn and appearance of skin, condition and moisture content, regularity in size, purity of race, and evidences of threshing damage; special care is needed in the examination of bulk samples on their arrival at the maltings.

It will be convenient to preface the description of methods of working in selecting barley samples suitable for malting by a discussion of modern barley varieties and methods of harvesting, since such topics are relevant to the more general problem. The choice of seed is difficult for the farmer. He usually knows whether his ground is suitable for Plumage-Archer or Spratt-Archer, as these were the only two barleys which assured him of top malting price from brewers before the war. War conditions, however, have resulted in Abed Kenia and Abed Maja being grown, in spite of warnings from the merchants that these varieties do not give the quality of barley that is required by the brewer. Abed Maja stands up better in adverse conditions than does Kenia, but the quality of the grain is poor. These Abed varieties are short strawed, and with the advent of combine harvesting plant and since they give an increased yield of 2 quarters *per* acre in some districts, their popularity with the farmer has risen. When controlled prices are removed, it is to be hoped that brewers will encourage the farmer to grow barley of a higher quality. Another new variety which has made its appearance during the war is Pioneer. This is a two-rowed winter-sown barley bred by crossing the German Tschermak's two-rowed winter barley with Spratt-Archer. It is a short straw winter-hardy barley of good malting quality. The grain is somewhat elongated, but during the last three seasons it has given an extract equal to that of any other variety. On the malting floor it is very active, and because of this must be malted

separately. Many farmers consider it a great advantage to sow one-third of their crop in the winter and have this harvested two weeks earlier than his spring-sown main crop; Pioneer makes such a plan an even more attractive proposition.

When purchasing seed, farmers buy top malting quality, grain therefore with the lowest nitrogen content—this is probably wrong. The young plant is largely dependent for its early supply of nitrogen on the endosperm of the seed, and it follows that those plants which are supplied with sufficient nitrogen by the parent seeds at this stage make the best start. In the subsequent competition, plants with adequate initial nitrogen supply will survive and those with inadequate supply will perish. A supply of nitrogen to a growing plant is as essential as a supply of moisture. E. S. Beaven (*Barley*. London: Duckworth, 1947) thought that, in this country, it would be better practice to sow a given weight *per* acre of large nitrogenous grains, rather than of small starchy ones. The whole of the nitrogen is not available for assimilation by the young plant; a considerable proportion of it is contained in the aleurone cells which are resistant to the enzymes which degrade the nitrogenous matter into assimilable forms, but the nitrogen of the starch-containing cells of the endosperm is broken down quite freely into food for the young plant. In order to grow barley of a suitable quality for seed Beaven considered that the sowing rate would have to be reduced to 56 lb. *per* acre on rich soils. This would yield a heavy crop of large grain, with a high

nitrogen content, coarse in appearance and of low malting quality, but it would have a higher value for sowing than barley grown under normal conditions from the point of view of good malting quality.

Assuming that the farmer has had suitable weather and his crop is ready for cutting—how is he going to harvest his crop? He can choose to (a) Thresh direct from stook; (b) Use a combine harvester; or (c) Stook, stack and thresh at his own convenience. To thresh direct from stook has many merits. It cuts out the high capital cost, or the expensive hire, of a combine harvester. Against that, ten days of good weather are required to allow the stooks to dry and then good weather is needed for threshing; unfortunately this spell of good weather only happens about once in 15 years.

The combine harvester method does much to rule out weather difficulties and to reduce labour, but while there are good points for this modern American method, there are also many drawbacks. The advantages for the farmer include:—

- (a) It reduces man-hours at harvest time when labour is particularly scarce.
- (b) Grain is secured in the condition in which it is cut, and does not stand out in the stook risking damage by weather or theft by birds and vermin.
- (c) The harvest is completely finished, and instead of threshing to do throughout the winter, labour is available for other work.
- (d) The farmer receives his money as soon as his crop is cut. This is a principal advantage.

The advantages for the maltster are:—

- (a) The crop is delivered at a time when malting operations are suspended and lofts are empty.
- (b) The grain has not sprouted in the stook.
- (c) The grain is wholesome and free from mould or "heated" corns.

Like most new methods there are always disadvantages; those which affect the maltster include:—

- (a) He has to dry the barley immediately it is delivered from the farm, as the average moisture is between 23–24 *per cent.* If this barley is not handled at

once, it will generate sufficient heat to injure or destroy the germ.

- (b) Each delivery has to be sampled and the moisture estimated. The arrangement for 1948/49 was that if the moisture exceeded 26 *per cent.* a drying charge was made; if under 25 *per cent.* then a cash adjustment only was calculated.
- (c) There is a greater proportion of extraneous matter such as straw, weeds, *etc.*, in combine harvested barley compared with stack deliveries. If the combine were fitted with perfect cleaning plant, the extra weight would cause the machine to sink into the ground. Thus screening is inadequate, and must be repeated prior to drying.

The one disadvantage from the farmer's point of view is that, at the beginning of the season, there will always be a flood of barley on the market and consequently a tendency for the price to weaken.

The third method of harvesting the barley crop, is first to stook the barley and then to stack it after 10 days. The farmer may then bide his time and thresh when the market is good. The maltster also gains by buying a drier and more mature barley for which he has not had to provide storage. This saves the maltster having to buy and store all his requirements at the beginning of the season, and he might consider it worth several shillings more *per* quarter to be able to purchase the barley as required.

It seems unlikely that the combine harvester will go out of fashion, though farmers may be forced to cut only part of their crop by this method, in order to avoid flooding the market and depressing the price.

To evaluate and to select the best and reject the poorer samples of barley offered in the market is a responsibility which calls for all the skill and experience the maltster has at his command. H. T. Brown has said (see A. J. C. Cosbie, this *Journ.*, 1944, 279):

"The one quality above all others which is required in a good brewing barley is facility for ready and complete modification on the malting floor; that is to say the starchy content of the endosperm must be reduced to a mealy consistency in order that the finished malt may possess sufficient friability and allow complete

permeation of the closely crushed material with water during the mashing process, so as to give full opportunities for the diastase to act on each individual starch grain."

That one sentence covers much ground although it was written many years ago, when malting quality was assessed on the physical characteristics of barley apparent to the eye and hand of an experienced maltster. It is now known that the buyer of barley for malting nearly always selects samples which subsequent chemical analyses show to be the "low nitrogen" barleys, and that prizes at barley competitions are almost invariably given to low nitrogen samples.

In the visual examination of samples the following points must be taken into account: ripeness, condition, moisture content, appearance of skin, regularity in size, shape, purity of race, presence of skinned and damaged corns due to faulty threshing.

The 1947 barley crop in some districts did not ripen normally. It "dried off," and the result was a sample with a "hungry" unfinished spindly appearance, and when corns were cut they appeared to be steely. In 1947 nothing could be done by the farmers to prevent this under-ripeness. The usual cause of an under-ripe barley is the farmers' fear of a break in the weather, his natural impatience, and the chance of loss due to birds or spillage making him cut his barley before it has ripened to the full extent. This practice of cutting grain before it is properly ripe is becoming more prevalent due to the use of hired combines. When the farmer arranges for the hire of a combine, he forecasts when the crop will be ready. In the meantime there might be a few weeks of sunless weather. The owner of the combine harvester sends his machine and men on the date arranged, and proceeds to cut the crop whether it is fully ripe or not. If the farmer stops the operation he will not later be able to hire a combine as the hirer must keep to his programme. The farmer may not be able to use his binder because of lack of labour; therefore in the end the maltster has to cope with immature grain.

The next point is the condition of the sample offered, which is decided by smelling. If it has a mouldy smell, it can be suspected that mould has developed in the stack, due to stacking in a damp state, or to faulty thatching. Thatching is intended to prevent rain entering the stack and is a

highly-skilled operation. The word "condition" in the case of combine barley does not mean mould but spontaneous heat generated in the sack, between the combine and the maltings. This is generally due to the grain being under-ripe and delayed in delivery before drying. The germ may then be damaged and there is no option but to reject the delivery. Instances have occurred of a complete motor load being rejected after lying in a warm garage (with a tarpaulin cover over the load) from Saturday mid-day until Monday morning. Sometimes the cause of combine harvested barley heating in the bags is due to an excess of green weeds, which may reduce the safe time limit from cutting to drying to 24 hours. The result of lack of condition in both cases means damage to the germ; if it is not entirely killed it will have a weak, sickly growth when it is malted.

Moisture content is not of great importance in combine barley as the price is adjusted, but if the sample offered from the stack is moist then the buyer is paying for water. If a sale sample from the stack is moist there will be a reasonable chance of part of the delivery being out of condition.

Next is the appearance of the skin, which is spoken of as thick or thin skinned according to its appearance. Actually there is no real measurable difference in the thickness of the husk, but the appearance of the husk is an indication of quality. The husk belongs to the leaf system of the plant and is not part of the seed itself; excessive manuring and other conditions which tend to produce luxuriant growth give, at the same time, coarse skin and large highly-nitrogenous corns which are lacking in mellowness. The testa, pericarp and husk are formed before the starch-containing cells of the endosperm. These develop from the centre outwards and exert great pressure on the envelope. The skins and husk consequently become stretched and smooth when the growth of the kernel is excessive but wrinkled on to the surface of the kernel when the growth is less luxuriant and this wrinkling is typical of a mealy grain.

Regularity in size is very important to both brewer and maltster. The maltster usually wishes to produce the maximum extract and can achieve this only if growth is stopped immediately starch conversion is complete. This conversion is more rapid

with small than with large grains, so that with irregular varieties a proportion of the corns will be either under-modified or overgrown, and extract will be lost. In the brewery a grist mill with multi-rollers can crush to an equal degree the small and large corns as they are actually graded before reaching the rolls. Unfortunately there are not many mills with this convenience; therefore the maltster has yet a further reason to be careful in selecting barleys as uniform in size as possible. Another problem is the shape of the corn. It is found that a plump barley will malt more satisfactorily than a large spindly barley, and also that the plump barley will be ready for the kiln before the spindly sample. The ideal would be to have sufficient loft capacity to allow Spratt-Archer, Plumage-Archer, Kenia and Maja barleys all to be stored separately, and then grade them all before steeping; the light barley would be sold for feeding. Doubtless the maltster who carried out this ideal practice would soon be seeking fresh employment. There is probably no maltings with sufficient loft space for one year's steeping requirements, so that it is unlikely that all the varieties now grown can be kept in individual parts, or lots in the lofts, and it is uneconomic to grade the barley severely at its present controlled price.

The damage to grain by threshing too closely is the last point to be considered when purchasing a sample. In the old days a barley with a bushel weight of over 56 lb. when delivered was subject to a bonus payment of 6d. *per* quarter, with another 6d. if it was over 57 lb. This principle was very good in that it encouraged deliveries free from extraneous matter, but the barley was chipped or skinned. Skinned barley readily produces mould which spreads quickly from corn to corn if the floor temperatures rise above 67° F. The other fault of threshing too closely is the tendency to have a high proportion of half corns which if not removed at the maltings will, like the skinned grain, produce mould on the exposed surfaces. It is true that half corns can be removed, but top price may have been paid for something that has to be sold at bottom price for pig feeding.

Delivery must now be dealt with. Acceptance of a sample is only the first step to receiving a delivery of barley, and with the best of intention on both sides purchase

samples and deliveries are rarely identical. Before the days of controlled prices farmers made a practice of keeping aside all damp sheaves from the top or bottom of the stacks. These sheaves were either fed to their cattle or threshed separately for pig meal; this is not the method now, when everything goes to the buyer whether it is damp or not. To combat this, it is necessary to have a man trained in the examination of barley to enable him to reject all bags unlike the sample purchased. The points he must look for are:—

- (a) Excessive quantity of light or broken barley.
- (b) Condition. This word is used to cover smell and dampness. It is very important that no mouldy or musty barley be accepted. This is usually visible to the eye by signs of a discoloured germ, but the eye is not nearly as sensitive as the nose. Sometimes germination tests have been carried out on lots that have been rejected for smell and the grain has given 100 *per cent.* germination. This is due to the fact that the grain is covered with mould spores which are detected by the nose, but the grain has not had sufficient time under adverse stack conditions to injure the germ. If grain with a strong mouldy smell is malted, then it is certain that the malt will be mouldy by the time it is ready to load on to the kiln. Barley is rejected for dampness because the maltster cannot afford to pay for excessive water.
- (c) Short weight in the bags by 2-3 lb. is not uncommon but the practice is to weigh three or four bags and if the average error is not more than one pound then no more bags are checked. The ideal method would be an automatic weigher at the hopper, but this is an expensive item and it is doubtful if all farmers would accept the weight without challenge.
- (d) A watch for stained or brown-ended corn is the last task of the man in charge of the intake hopper. There are two kinds of brown ends. The first has a reddish-brown colour and is the result of "heating" or spontaneous combustion in the stack due

to stacking the sheaves in a raw condition. This may be brought about by rapid mould growth along with bacterial growth, and the result is a grain which will not germinate. The other stain is of a grey-brown colour and is in no way injurious to the germ as it arises when the grain is in the ear. This is noticeable in the 1948 crop which was harvested under abnormally wet conditions. It is interesting to dwell on this point more fully. When barley begins to ripen, the ear bends over from an erect position to become nearly parallel with the straw, but one or two of the upper grains do not bend over with the rest of the ear but remain erect. The dew and rain collect at the angle formed by the grain and the rachis. A globule of moisture is formed which stains the one or two grains and so makes them appear dark ended. The stain is still noticeable after malting and sales maltsters dislike it because the buyer of malt may suspect "heated" barley as being the cause. Actually this type of stain does not effect the germination.

The barley, having passed the checker's nose, eyes and hands, has now to be dried to a moisture which will make it possible to store the grain for several months. In Scotland the moisture of barley from stack averages 18 *per cent.* and it is usual to dry down to 10-12 *per cent.* This drying improves the growth and finishes off the ripening of the dour Scotch barley which is famous for its dormancy, as well as its large extract. The problem of dormancy has only now become serious because under present restrictions maltsters do not have a large carry-over and require a barley that will malt soon after harvest time, so that an early start may be made in the maltings. From the number of burst and sprouted grains experienced during the 1948/49 season's deliveries it may be thought that the grain is not dormant enough in a wet harvest. Various theories about dormancy have been published (see, *e.g.*, *J. Incorp. Brewers' Guild*, 1947, 33, 189), but existing theories do not satisfactorily explain the problem. For instance, consider the idea that grain is worst if harvested in a wet

season. The 1947 crop which was harvested in the best weather conditions which farmers can remember was more dormant even after nine months' storage than the 1948 crop eight weeks after kilning. This may be explained by the tremendous amount of skinned or damaged grain in the 1948 crop. The latter crop was over-ripe before the combines were able to commence and the skinning probably allowed oxygen to reach the critical part of the germ without interference. It is not suggested that farmers should be encouraged to market skinned corns; it is true that germination is encouraged, but trouble is likely to arise with moulds if the grain temperatures rise above 65° F. on the malting floor. When skinned corns are present it is always advisable to wet 10 *per cent.* less than normal in each steep. This gives a safety margin should the weather be unsuitable and will restrict high grain temperatures on the malting floor, so restraining mould growths.

The drying of barley can be carried out in malt kilns, in revolving drums or in any form of continuous driers. The same result can be obtained by each method if the plant is properly constructed and controlled. Grain is a bad conductor of heat, so that when hot air is applied to wet grain the heat does not penetrate easily and there is a consequent drying of the surface with shrinkage of the skin, which hinders the drying of the moisture from the interior. Water, however, is a much better conductor of heat, so that if grain can be heated up in a wet atmosphere the heat will penetrate along the moisture channels of the grain and gradually assist in providing uniform heating. The revolving drums are always called "sweating" drums, and never "drying" drums. If they had been meant for drying quickly then they would have been fitted with much more powerful fans. This problem arose with continuous driers but was overcome by fitting a chamber with hot air coils excluding all air from passing through this section. In other words, the grain is first sweated, and the temperature brought up to 105° F. before any drying is carried out. The continuous driers are used mainly by commercial driers in Scotland, and at first were not approved by maltsters. If these driers are operated by a trained staff then there is no doubt that results will be as good as in the older method of kiln drying. The

wisdom of encouraging farmers to install continuous drying plant to handle the output of combines is doubtful; careful and uninterrupted attention is essential if risk of fatal injury to the germ is to be avoided.

The storage of dried barley is becoming a serious problem now that the combine harvester is flooding the market with say 35 *per cent.* of the total crop in a period of four weeks. Commercial drying companies have helped to dry combined barley but they are using a large amount of outside storage which would otherwise have been available for the maltster. Dried barley may be stored in empty malt bins always available at the beginning of a season, but it is necessary to install thermometers at various levels and to provide facilities for turning the grain from one bin to another. Even if the temperature does not rise it is still necessary to aerate the grain every four

weeks to get rid of the carbon dioxide produced during storage. Ten years ago it was the recognized practice to store dried Scotch barley to a depth of 36 inches but while this is ideal it has been found necessary almost to double this limit. All maltings unfortunately are not constructed to take this additional weight, and all maltsters should have their lofts examined to determine the maximum load the lofts will carry safely. If grain is stored at say 6 ft. in depth, it is necessary to turn it by dropping after about 5 or 6 weeks if the grain is to be kept sound. Where dropping is not possible it is useful to have a small suction plant to turn the grain over, thereby saving many man-hours as compared with hand turning.

Edinburgh.

January, 1949.

MEETING OF THE MIDLAND COUNTIES SECTION HELD AT THE WHITE HORSE HOTEL, CONGREVE STREET, BIRMINGHAM, ON THURSDAY, 20TH JANUARY, 1949.

Mr. B. WOOD in the Chair

The following paper was read and discussed:

THE DESIGN AND INTERPRETATION OF TASTING EXPERIMENTS

By C. J. VIRDEN, M.A., B.Sc., D.Phil.

Factors in the design of valid tasting experiments, and in the selection of a representative tasting panel, are discussed, emphasis being laid on replication, independence of results, and reproducibility. Pairs tasting technique, to elicit public preference for one of two samples, is described in some detail, and practical aspects of interpretation of results are considered. Triangular tasting is only useful to detect differences between samples and is best carried out by a panel selected primarily for tasting ability. Test has also been made by noting from day to day over a long period which of two beers was selected at lunch by a group of people. Again, after giving two beers on alternate days for four days, free choice may be allowed on the fifth. It is noted that drinking and tasting experiments do not necessarily give the same results.

EARLY in 1946 a paper was read by Dr. Erik Helm of the Carlsberg Breweries on the subject of sample tasting (this *Journ.*, 1946, 165). This important paper was the first publication in the English language dealing with the technique of the examination of beer by tasting. In this paper Helm described experiments which he and his

colleagues had carried out, designed to investigate the best way of detecting differences in flavour by tasting.

In this paper the subject will be approached from a rather different point of view. It is proposed to give some account of the way in which the problem of taste-testing has been dealt with in a particular brewery, and

while it may be felt that the solutions presented are not such as will be applicable in the varied circumstances of different brewing firms, a discussion of the factors involved may still be helpful. The first point to be made is that the designer of a tasting experiment usually is concerned to estimate which of two beers will be preferred by the public who consume the beer. It naturally follows that if one of two beers is preferred there must be a difference between them. The converse, however, is not true. Two beers may be different without there being a marked preference for one of them. Therefore, the designer of a tasting experiment may legitimately design the experiment to answer either of these questions:

- (a) Which of these two beers is liked best?
or
- (b) Are these two beers detectably different by taste?

These two questions are not equivalent to one another. It is submitted that, for many purposes, it is essential that the first question should be answered—that is—“Which of these beers is liked best?”

At this stage it is as well to consider what methods are open to the brewer in designing his tasting experiment. He may use the triangular method of tasting examined in such detail by Helm, but this technique is only capable of answering the question—“Is there a difference between these two beers?”; it is fundamentally incapable of saying which of the two beers is liked best. The next most hopeful technique is to offer to tasters the beers in pairs, asking the question—“Which of these two beers do you prefer?” The answers to this question can form the basis of an estimate of the relative popularity which the two beers are likely to enjoy, but only if the tasting panel represents with reasonable accuracy the tastes of the public who are going to consume the beer. This latter proviso is really of fundamental importance. The tasting panel is a sample of the public and it must represent that public as accurately as possible. Therefore great pains must be taken to select a panel which is as representative as possible in order to answer this question—“Which beer will the public like best?” Every type of palate ought to be included in its proper proportion. Obviously it is very difficult to achieve this

ideal, but there is no doubt at all that something much better than the small panel selected—very often exclusively—from the brewing staff can be achieved by drawing representatives from every part of the brewery. However, it must be realized at this point that no tasting results can be more representative of the truth than the panel is representative of the public.

The next point, and one which arises naturally from what has passed before, is the avoidance of all possibility of prejudice. The people carrying out the tasting trial should, preferably, have no idea at all beforehand what the experiment is about. They ought to be presented with two samples of the beer and asked the question—“Which of these two do you prefer?” Furthermore, reasonable precautions should be taken to prevent one taster from influencing the decision of another.

If these precautions are all observed, the human side of the tasting trial will have largely been dealt with. There is another side, however. Very often the brewer has to make a decision about the relative merits of two processes or two materials, which, in the very nature of things, cannot be expected to make much difference to his product. Under these conditions it is especially important that he should realize the necessity for adequate replication of his experiment. Thus, suppose the point being examined is—“Which of two types of 2-rowed barley makes the best beer?” It is quite likely that the difference in quality of the two beers will be small compared with the normal variation in the brewing process and, under these circumstances, it is essential that an adequate number of replications of the actual brewings should be included in the tasting trial. This is really another aspect of the same point that was raised over the choice of the tasting panel. The brewings tasted must represent adequately the general run of brewings and must not be highly specialized single brewings.

There are, therefore, two important criteria which must be fulfilled, *viz.*, that the tasting panel must represent the public and the brewings being tasted must represent the general run of beer which could be produced in the brewery. In addition, certain precautions must be observed to avoid undue influence—what has aptly been called “sheep

effect"—which occurs when one person influences the opinions of others.

Under these conditions the tasting experiment may now be begun. The two beers should be set out under some code numbers and the tasters should be invited to express their opinions. Experience indicates that it is difficult to express valid opinions about more than six samples at any one time. That is to say, it is reasonable to ask people to state their preferences in each of three pairs of samples, but no more. In setting out the samples for tasting it is necessary that they should not appear in systematic order; thus, when the two treatments are A and B, treatment A should appear on the left hand side as frequently as it appears on the right hand side. The reason for this is that individuals tend to pick up the glasses in the same order always and taste them in the same order always, and many people find that they have a predisposition in favour of the beer which they tasted either first or last. It is important to balance out this effect.

When the results are finally collected they will be in the form of a number of preferences for A on the one hand and a number of preferences for B on the other. At the same time a certain number of tasters may have refused to express any preference at all. There will be, therefore, a certain number of "no preferences." It is desirable to keep this number of "no preferences" as low as possible. Tasters should also have been invited to make comments giving reasons, if possible, for their preferences. This is a very important side to the whole trial, for it is sometimes possible to show by an examination of such comments that a certain proportion of the tasters prefer the beer A, for example, because it was more bitter than B, while the rest of them prefer B because it was less bitter than A. Thus, while over all there is no preference for A over B, it may be clear that Treatment A produces a beer more bitter than B.

While so far preferences only have been discussed, there is no reason why tasters should not be asked more than one question. Usually one of these questions will be—"Which do you prefer?"—but they can at the same time be asked other questions such as—which beer is more bitter, or more acid, or any other question which may interest the experimenter. Experience shows that it is

usually best to confine the number of questions to 3 or 4 at the very outside.

Next comes the interpretation of the figures which have been obtained. Here it is essential to have some understanding of the statistical method of analysis which is involved. The problem is something like this—suppose that out of 60 tastings of A versus B, 37 people have preferred A. The question which has to be answered is—"Does this imply a real preference for A or is this just a chance variation?" Fortunately this type of question can be answered relatively easily. The line of argument is as follows: if there is no real preference for either of these beers then the probability that any particular beer will be chosen on any particular occasion is one-half. The probability that the other beer will be chosen is similarly one-half. Under these circumstances it can be shown fairly easily that if a certain number N , in this case 60, trials are made, the frequency with which the various different possible numbers of successes are recorded would be distributed in accordance with the terms of the expansion of $(\frac{1}{2} + \frac{1}{2})^N$. It is possible to calculate all these terms although it is somewhat laborious. If this is done it will be found that on approximately 4.7 *per cent.* of occasions when 60 tasting trials are carried out there will be 37 or more preferences for one of the beers tasted even though there is no real difference between them. Expressed differently this says—there is a 4.7 *per cent.* probability of getting the observed result by chance even if there is no difference. This order of probability is usually regarded by statisticians as being on the lower limit of significance. This means that statisticians are usually unwilling to make any important decisions unless the results they get can have been obtained by chance with a smaller probability than this. For example, if 40 preferences for one of the beers had been observed out of 60 trials such a result could be observed by chance with a probability of only 0.7 *per cent.*, while 45 preferences for one could only have been observed by chance once in about 10,000 trials; that is to say, it is virtually certain that the difference between the two beers is a real one and, under these circumstances, a prudent man would be entirely justified in modifying his process in the direction indicated by the results of the experiment.

It is important to notice that this method

of analysis is only valid if the conditions which have been laid down earlier are observed. That is prejudice and "sheep effect" must be absent as must all extraneous influences; in other words, all the opinions recorded must be independent genuine expressions of preference based only on the merits of the beers. Though really obvious enough, this is perhaps sometimes forgotten.

Naturally, the laborious method of analysis indicated above is not generally employed. Much easier is the χ^2 test referred to by Helm. Details of the application of this test are to be found in any of the text-books of statistics.

This aspect of the pairs tasting experiment has been dealt with at some length because it seems most important that tasting trials carried out by brewers should be done as reliably as possible and that the most prudent decisions should be taken in the light of the evidence which is available. This may be illustrated as follows: suppose that tasting trials have been carried out to establish whether or not a new variety of hops is a suitable brewing material, with tasting results based on an adequate number of different brewings and on an adequate number of different tasters' opinions. A variety of different answers can be imagined: (1) Tasting shows that the new variety of hop is only preferred on 10 *per cent.* of occasions while the old variety is preferred on 90 *per cent.* of occasions and, from the number of tastings the probability of getting these results by chance is very small, let us say one in 10,000. Under these circumstances no one has any hesitation in rejecting the new variety of hops. (2) The opposite result might be obtained, *viz.*, the new variety is preferred on 90 *per cent.* of occasions with an equal probability of observing this result by chance. Again no one would have any hesitation in accepting the new variety of hop. These are the easy cases. Of much more frequent occurrence are the borderline cases. (3) The case where there is a slight but not statistically significant dislike of the new hop. Under these conditions the brewer certainly ought not to accept the hop. He should decide on other grounds whether it is worth his while doing further experiments to establish whether this slight dislike of the hop is a real phenomenon or not. The other grounds might be cheapness, or high preservative value, or ready availability of the hop.

(4) In the fourth case a slight but insignificant preference for the new hop might lead to a similar answer but perhaps the brewer would be willing to use this new variety without requiring further experiment depending on other considerations.

This leads to one final but very important consideration. The importance of replication has been emphasized. Now consider a particular type of experiment, one which has been carried out by a number of brewers recently. The question is asked whether casks made of a new variety of wood or other material are suitable containers for beer. The brewery obtains a cask of, *e.g.*, Ruritanian oak, and uses it on a number of occasions carrying out well designed tasting trials, which demonstrate, let us say, a slight but significant preference for beer drawn from the Ruritanian oak cask. The careless would be apt to say that this proved conclusively the suitability of Ruritanian oak for beer containers, but a little further consideration will show that this is not so. All that this series of experiments does, in fact, demonstrate, is that that particular cask is a suitable container for beer and it is certainly not justifiable to extrapolate from this one case to all other casks made from Ruritanian oak, from different trees, felled and handled under different conditions. It is essential, in order to get reliable results in a case like this, that oak from different sources, different trees and so on should be obtained and used under experimental conditions. It is impossible to lay down how many independent trials must be made but, in that type of case, where strict independence can be assured, three concordant trials will give reasonable presumptive evidence that the experimental material is satisfactory. It is important to notice that the strict independence of the results and the concordance of the results has been emphasized. If either of these factors is under suspicion, more than three trials *must* be carried out.

The triangular tasting technique has already been dealt with very fully by Helm. However, even there, there are certain precautions which ought to be observed. For example, as was implied earlier, the day to day variability of the process may interfere with the validity of tasting trials. When the triplet type of tasting is, for example, used to find out whether the introduction of a new brewing material has caused a

difference to the beer, it is important that any difference that is detected should be strictly attributable to the factor being examined and not to the ordinary day to day variability of the brewing process. To some extent this can be ensured by the method of tasting to be described. Suppose brewings are carried out in which A represents the new factor and B represents the old factor which A replaced. Brewing A1 is the first brewing in which the new factor is introduced. Brewing B1 is the first of the experimental series where the old factor is retained. We then brew in the order A1, B1, B2, A2, A3, B3, B4, etc. The brewings are then set out for tasting in triplets, tasting A1 with B1 and B2; B1 and B2 with A2. B2 with A2 and A3 and so on. In this way the following advantages are obtained: (a) the average age at which each beer is tasted is nearly the same, and (b) since on any one occasion the difference between the odd one and the other two must be greater than the difference between the other two for a correct result to be given, any significant result can be attributed to the treatment and not to day to day variability.

Another aspect of triplet tasting which is worthy of consideration is that this technique is only useful in detecting differences without regard to whether the difference is desirable or otherwise. That is to say, in no case can the results be extrapolated to indicate the popularity or otherwise of a particular beer being acceptable to the consumer. Therefore since the results cannot and will not be applied as predictions of public taste it is unnecessary that the tasting panel should be representative of the public and indeed, in this case, the best results are obtained by carefully selecting members on the basis of their tasting ability. At the same time it still remains necessary to replicate adequately the number of brewings tasted.

At this point it might be of value to give some indication of the numbers of brewings and tasters desirable. In the very nature of things it is not possible to lay down beforehand what numbers of replications are required since this depends on the variability of the process. However, it is unlikely that misleading results will be obtained if every experimental series contains 12 replications. As far as numbers of tasters are concerned, for pairs tastings for preference obviously the

larger the number the better. They represent a cross section of the public and probably it would be well never to have less than 40 or 50 individuals though this is an ideal difficult of attainment. As regards the triangular tastings where differences only are being observed, small numbers can be a positive advantage and perhaps half a dozen skilful tasters will produce more information more quickly than any number of less skilful.

The next type of experiment is one of which little experience is available, but, nevertheless, it may prove to be more important than any other. In the ordinary way much tasting is done by the taster taking a sip or two of the beer which he may or may not swallow and basing his opinion on this. This, of course, is a rather artificial proceeding but one which is enjoined upon us by the fact that a good many samples have to be tasted in the course of a day, and even in the course of a relatively few minutes. Much more convincing are results obtained from a tasting trial in which each of the tasters drinks some reasonable quantity, say a half-pint. This type of experiment can be organized in one of two ways.

The first technique is one in which a group of people are asked to choose which of two different beers they will drink with their lunch, with the proviso that they can only have one and not both on any one occasion. The results of this choice are recorded from day to day and form the basis of the estimate of the probable relative popularity of the two beers. As in the pairs tasting technique where the question asked is—"Which do you prefer?" it is of first class importance that the panel should represent the public as closely as possible and 40 or 50 is a minimum number desirable for getting reliable results. The experiment has to be continued for some considerable time and probably needs to be repeated at different seasons and under different conditions. The results can then be expressed in the form that on each day a certain proportion of the total beer consumed was variety A or variety B. If this proportion is close to one half there is obviously little or no difference in the appeal of the beer to the palate and the further the proportion gets from one half, the greater is the difference. There is, unfortunately, no method of statistical analysis which can be applied to these figures which will assess their reliability. The reason for this is that

the results of one day are necessarily influenced by those of the preceding days and therefore the results do not possess the necessary quality of independence to allow them to be analyzed statistically. Despite this drawback this type of experiment is of very great value. The results must be examined in order to see whether there has been any systematic change in the preference for one beer or the other and from this examination the experimenter can form some opinion as to whether a stable state has been reached. If it has not been reached, clearly the experiment must be prolonged until it has. It is interesting to note that this type of experiment does not necessarily give the same results as a more normal tasting experiment. The following type of result has been observed:

	Percentages.	
	By drinking.	By tasting.
Preference for A ..	82	51
Preference for B ..	18	49

In this case the drinking experiment showed a very marked preference for one beer, which preference was not detected significantly by an ordinary tasting panel. On another occasion the tasting test as opposed to the drinking test gave a result in the opposite sense. Since the public buy our beer to drink and not to taste there is no doubt which of these results is the more valuable.

A second technique for this type of drinking experiment has been tried. In this the drinking panel has been given the two beers on alternate days for, say 4 days. On the fifth they are asked to choose which they will drink. This must be repeated on an adequate number of occasions and the labelling of the beers should be changed on each occasion so that there is no danger of the results of one experiment being influenced by those of the previous one.

This particular arrangement of experiment possesses the advantage that the same type of statistical analysis can be applied as is applied to the ordinary tasting experiment and therefore the reliability of the results may be assessed.

This type of experiment has been carried out on a number of occasions and the results

can again be compared with the results of a more normal tasting experiment for preference. Just as in the other drinking experiment the same results are not necessarily obtained by the two techniques. Thus on one occasion the following results were obtained:

	Percentages.	
	By drinking.	By tasting.
Preference for A ..	51	25
Preference for B ..	49	75

By drinking there is no significant difference between the two beers whereas by tasting a very marked and highly significant preference for one of them is observed.

From this small number of examples it is clear that the technique by which an assessment of the quality of beer is made, that is, by drinking or by tasting, will have a very marked influence on the results observed. There is little or no doubt that either of the drinking techniques is likely to give a result more in conformity with the opinion likely to be formed by customers than is the tasting technique. This point has then been reached: while perhaps the ordinary tasting technique can be a valuable and informative guide to the likely reactions of people to beer under trade conditions, it can be no more than this, and more reliable results can perhaps be obtained if the beer is tested by drinking it under conditions which approximate more or less closely to those which obtain in real life.

This last type of experiment has only been tried recently, and therefore it is not possible to lay down precise conditions in which the best and most reliable results are obtained. Further work, however, on these lines may show the pitfalls and difficulties which must be avoided.

The author wishes to express his thanks to the Directors of Messrs. Arthur Guinness, Son & Co., Ltd., for permission to publish this paper, and also to those of his colleagues who collaborated in carrying out part of the work described.

Park Royal Brewery,
London, N.W.10.

COMMUNICATIONS

TWO NEW HOPS RESISTANT TO *VERTICILLIUM*-WILT*

By Prof. E. S. SALMON

The characters of the new hops are described in detail. They are symptomless carriers of mosaic disease, but are more susceptible to Downy Mildew than is Fuggle. They have a P.V. higher than in ordinary commercial varieties, and brewing trials carried out in 1945 and 1948 showed that, in 11 out of 13 breweries, they were probably acceptable as brewing materials when used in 10-30 per cent. blend with other hops. Rooted sets are available.

THE two new varieties of hops described below *Ref. Nos.* OR55 and OJ47 were raised at Wye College, in 1924 and 1930, respectively; both are partly of American ancestry. After being grown and studied at Wye College, they were in due course sent, with a large number of other new varieties, to the East Malling Research Station, where during recent years Mr. F. H. Beard has collected data as to yield, cultural features and susceptibility to disease. In 1938 Dr. W. G. Keyworth commenced his studies of the very dangerous disease *Verticillium*-Wilt (progressive form) caused by the fungus *Verticillium albo-atrum* Reinke and Berth. In his tests during succeeding years, using all the ordinary commercial varieties and the large number of new varieties assembled at East Malling Research Station, Dr. Keyworth discovered that the varieties OR55 and OJ47 are, to use his words, "moderately resistant" to *Verticillium*-Wilt. For information as to the nature of this resistance the reader is referred to the various articles which have been published by Dr. Keyworth (see Bibliography, below).

The two varieties have many characteristics in common. Both are strong growers; a "plant" of 7 ft. by 7 ft. has been found satisfactory at East Malling and is recommended, as with closer planting the danger is increased of severe attacks of Downy Mildew. Wirework of ordinary height is suitable for OJ47, though possibly OR55 will benefit from slightly higher wirework; two bines are required to each string. Both varieties are immune from the effects of mosaic disease, since they carry the virus without being affected in any way. Varieties such as these (which are termed "carriers")

should, therefore, not be planted near to mosaic-susceptible varieties (Goldings and "Golding Varieties") in view of the possibility of their infecting the latter. On the other hand, they may safely be planted adjoining the Fuggle, as this variety is likewise a "carrier" of mosaic disease.

Both varieties are more susceptible to Downy Mildew than the Fuggle, resembling in this respect the Goldings and "Golding Varieties." They are very liable to late infection of the upper laterals just before and during the burr period, if weather favours the disease; OJ47 is perhaps the more liable of the two to the production of basal and lower lateral "spikes." Consequently it is essential in order to obtain healthy growths of both varieties, that all basal, terminal and lateral "spikes" are promptly removed throughout the season and that the necessary sprayings with a copper-containing fungicide are given. Damp situations, or adjoining woodland, should be avoided; otherwise extra precautions against Downy Mildew should be taken.

As shown below in Tables II and IV, both varieties are rich in soft resins, with a preservative value (P.V.) higher than that found in ordinary commercial varieties.

In the brewing trial made in 1945 (see Bibliography, 4), and also in the trials made in 1948 in 13 breweries in various parts of England and also in Scotland, either OR55 or OJ47 or both varieties were found, in 11 out of 13 cases, to be probably acceptable in blends of from 10 per cent. to 30 per cent. with other hops.

In 1948 a Committee appointed by the Hops Marketing Board arranged for the propagation of both varieties, on a large scale, to be undertaken, so that rooted sets should become available for growers who

* The Report now reprinted was published in January, 1949, by Wye College, Kent. Price 2s. 6d.



FIG. 1

"Keyworth's Midseason" (*Ref. No. OR55*)
Upper lateral branch of Hops, $\frac{1}{2}$ nat. siz. Wye, Sept. 7th, 1948



FIG. 2
"Keyworth's Midseason" (*Ref. No. OR55*)
Six cones, nat. size. Wye, Sept. 7th, 1948



FIG. 3

"Keyworth's Early" (Ref. No. OJ47).
Upper lateral branch of Hops, $\frac{1}{2}$ nat. size. Wye, Sept. 3rd, 1948



FIG. 4
"Keyworth's Early" (*Ref. No. OJ47*)
Six cones, nat. size. Wye, Sept. 3rd, 1948

wish to plant up areas where, owing to soil infestation by the *Verticillium* fungus, the cultivation of the Fuggle and other commercial varieties has become impossible. Applications for sets should be made to the Secretary, Medway House, High Street, Maidstone.

A detailed description is given below of each variety, together with data extracted from the *Reports on the Trial of New Varieties of Hops* (see Bibliography, 5).

It is proposed to give the name of "Keyworth's Midseason" to OR55 and "Keyworth's Early" to OJ47.

"KEYWORTH'S MIDSEASON" (Ref. No. OR55)

Parentage. This seedling was raised in 1924 from an "open" flower of the seedling Ref. No. Y90, raised from an "open" flower of the American wild hop (*Humulus americanus* var. *neo-mexicanus*), growing in the Nursery at Wye College.

Botanical Characters. Bine green, with faint green longitudinal lines; upper laterals of medium length with the cones more or less densely clustered, lower laterals longer; cones pale green, when fully ripe with a golden tint, oblong or subcylindrical, firm, with very numerous "petals" (*bracts* and *bracteoles*), $1\frac{4}{10}$ in. to $1\frac{9}{10}$ in. long, $\frac{8}{10}$ in. to 1 in. wide, tip rounded, more or less closed; strig green, thick.

This is a late-midseason variety, of vigorous growth, and heavy yield; the cones are of fair size, "bunchy" and easy to pick. It is slightly less susceptible to Downy Mildew than "Keyworth's Early" (Ref. No. OJ47), but more so than the Fuggle and requires a thorough control programme. Photographs of an upper lateral branch and of six cones are reproduced in Figs. 1 and 2.

TABLE I

Year.	No. of Imperial Bushels of Green Hops to cwt. Dried Hops.	Estimated yield per acre.
1940	82	18
1941	109	19½
1942	80	22½
1943	83	25½
1944	107	18
1945*	—	—
1946	85	17½
1947	93	22½

* Crop not recorded owing to attacks of "mould."

Yield. Table I gives the estimated yield per acre of the row of fifteen hills at East Malling Research Station from 1940 to 1947, and the number of imperial bushels of green hops to the cwt. of dried hops.

The low number of bushels of green hops required to the cwt. of dried hops is due to the firm, dense cones composed of very numerous "petals" and to their richness in soft resins.

Aroma. In 1934 Mr. C. G. Tosswill reported on a sample grown at Wye College: "'Manitoba' aroma, but not pungent." In 1946 Col. F. N. Richardson and Mr. A. E. Quin reported on a sample grown at East Malling Research Station: "Good appearance, thick side; 'Manitoba' aroma, but not rank."

Preservative Value. In 1935 an analysis, made on 25th November, on a sample grown at Wye College, gave, on moisture-free hops 7.85 per cent. α -resin, 9.55 per cent. β -resins.

Table II gives the chemical analyses (on moisture-free hops) of the growths at East Malling Research Station from 1941 to 1947.

TABLE II

Year.	Date of analysis.	α -acid per cent.	β -resins per cent.	P.V. (10 α).
1941	12.1.42	5.33	9.55	53
1942	—	—	—	—
1943	7.3.44	7.20	9.19	72
1944	18.1.45	8.68	8.00	87
1945	—	—	—	—
1946	28.10.46	6.70	7.29	67
1947	28.10.47	8.15	8.81	82

"KEYWORTH'S EARLY" (Ref. No. OJ47)

Parentage. This Seedling was raised in 1930 from an "open" flower of the Seedling Ref. No. EE92. EE92 was raised in 1920 from an "open" flower of the Seedling Ref. No. Y90, raised from an "open" flower of the American wild hop (*Humulus americanus* var. *neo-mexicanus*) growing in the Nursery at Wye College.

Botanical Characters. Bine green, with dark green longitudinal lines; upper laterals short with densely clustered cones; cones grass-green, firm, somewhat variable in size and shape, ovate, ovate-oblong or oblong-cylindrical, $1\frac{4}{10}$ in. to $2\frac{1}{10}$ in. long, $\frac{9}{10}$ in. to 1 in. wide, usually ovate-oblong,

1 $\frac{6}{10}$ in. to 1 $\frac{7}{10}$ in. long, "petals" (*bracts* and *bracteoles*) very numerous, tip of cone rounded, more or less closed; strig green, of medium thickness.

This is an early variety, vigorous and fruitful, with large, clustered cones which are easy to pick. The cone is occasionally somewhat "open" or "ragged" in its lower part (when seeded), due to the *bracts* standing apart; when, however, the cones are more regular and oblong-cylindrical, there is a "family resemblance" with "Keyworth's Midseason" (*Ref. No. OR55*). The cones of the upper laterals not infrequently produce leaves. Photographs of an upper lateral branch and six cones are reproduced in Figs. 3 and 4.

This variety is more susceptible to Downy Mildew than "Keyworth's Midseason," and should not be planted unless a thorough control programme can be carried out.

Yield. Table III gives the estimated yield *per* acre of the row of sixteen hills at East Malling Research Station from 1940 to 1947, and the number of imperial bushels of green hops to the *cwt.* of dried hops.

TABLE III

Year.	No. of Imperial Bushels of Green Hops to <i>cwt.</i> Dried Hops.	Estimated yield <i>per</i> acre.
1940	94	18 $\frac{1}{2}$
1941	80	18 $\frac{1}{2}$
1942	92	21 $\frac{1}{2}$
1943	90	21 $\frac{1}{2}$
1944	95	23 $\frac{1}{2}$
1945*	—	—
1946	99	10 $\frac{1}{2}$
1947	98	27 $\frac{1}{2}$

* Crop not recorded owing to attacks of "mould."

The very low number of bushels of green hops required to the *cwt.* of dried hops is due to the firm, dense cones composed of very numerous "petals" and to their richness in soft resins.

Aroma. In 1934 Mr. C. G. Toss will reported on a sample grown at Wye College: "Good appearance and rub; strong 'Manitoba' aroma. A really good hop." In 1946 Col. F. N. Richardson and Mr. E. A. Quin reported on a sample grown at East Malling Research Station: "Good appearance, very thick side, strong unpleasant 'Manitoba' aroma, good rub."

Preservative Value. Table IV gives the chemical analyses (on moisture-free hops) of the growths at East Malling Research Station from 1943 to 1947.

TABLE IV

Year.	Date of analysis.	α -acid <i>per cent.</i>	β -resins <i>per cent.</i>	P.V. (10 α).
1943	8.11 to 20.12.43	7.62	9.12	76
1944	18.1.45	7.20	7.30	72
1945	—	—	—	—
1946	1.11.46	6.74	8.61	67
1947	4.11.47	5.58	9.47	56

ACKNOWLEDGMENTS

Our thanks are due to Mr. F. H. Beard for much valuable information, and to Mr. H. H. Glasscock, M.Sc., N.A.A.S., for the photographs reproduced in Figs. 1 to 4.

BIBLIOGRAPHY

1. Keyworth, W. G., "Verticillium-wilt of hops." *Brewing Trade Rev.*, 1947, **59**, 100.
2. Keyworth, W. G., "Verticillium-wilt of the Hop (*Humulus lupulus*). II. The Selection of Wilt Resistant Varieties." *J. Pomol.*, 1947, **23**, 99.
3. Keyworth, W. G., "Notes on Varieties of Hops resistant to *Verticillium*-wilt." *Rep. E. Malling Res. Station for 1946.* 1947, 157.
4. Thompson, L. C., and Brown, B. M., this *Journ.*, 1945, 39.
5. Salmon, E. S. Twenty-fourth to Thirty-first Reports on the Trial of New Varieties of Hops, 1940-47, this *Journ.*, 1941, 371; 1943, 9; 1944, 28, 270; 1945, 224; 1946, 294; 1948, 7; 1949, 29.

ELECTROLYTES IN STARCH STRUCTURE AND THEIR BEHAVIOUR IN THE HYDROLYSIS OF STARCH BY BARLEY AND MALT AMYLASES

By Dr. H. LE CORVAISIER

It is argued that the problem of the duality of barley and malt amylases is not resolved, at least in the sense that there exist distinct α - and β -amylases producing oppositely muta-rotating maltose. From a study of starch liquefaction and saccharification, chiefly with malt amylase, the actions of a liquefying and a saccharifying enzyme may be recognized; however, the two functions are not clearly distinct, and the amylolytic complex in malt is not essentially different from that in barley. The second enzyme may be a phosphatase, but its action is not entirely individual since it acts with the amylase itself. Linear starch molecules (little esterified) would be hydrolyzed by the amylase (saccharification); esterified chains would be hydrolyzed by the joint effect (liquefaction) of amylase and perhaps phosphatase, the organic phosphates being degraded and sugar liberated.

In a recent publication (le Corvaisier, *Bull. Soc. Chim. biol.*, 1948, 30, 202) it is said that the starch granule is composed of homogeneous glucosic material associated with mineral matter, the degree of condensation varying with the nature of the starch and the size of granule. The mineral matter is chiefly phosphate, which plays a part in starch synthesis and possibly also in the hydrolysis of starch pastes. The kations and even the anions (including phosphate) can be partially removed from the granule by washing with water; hence it appears that part of the inorganic material is only adsorbed by the granule. In pastes, however, inorganic material is probably combined to the glucosic material by 1-6-glucose ester linkages, chiefly in the branched molecules.

Again, it is not considered that amylose and amylopectin (or to use terms which do not prejudice the constitution of the starch molecule, the fractions A and B of T. J. Schoch, *Advances in Carbohydrate Chem.*, 1945, 1, 247) exist as such in the granule. In view, in fact, of the heteropolar nature of starch it is considered that the paste is a product of depolymerization. Recently, A. Macey (*Comm., VII Congrès Internat. Ind. Agric., Paris*, 1948) has discussed methods for passing from straight to branched chain polysaccharides by the action of two enzymes, the actions of these phosphorylases being very different from those of amylases (cf. C. S. Hanes, *Proc. roy. Soc.*, 1940, 129, 174; E. J. Bourne, A. Macey and S. Peat, *J. chem. Soc.*, 1945, 882). Again P. Bernfeld and M. Fuld (*Helv. chim. Acta*, 1948, 31, 1420, 1725) have isolated a phosphorylase and an

isophosphorylase from potato juice and have shown how to secure complete hydrolytic degradation by combining the action of phosphorylases (which they considered to be free from phosphatase) with that of α - and β -amylases.

It may be recalled (le Corvaisier, *loc. cit.*) that the removal of the electrolytes from paste takes place principally during liquefaction and in the form (according to Posternack) of phosphoric esters in the case of potato starch, and in view of the importance of phosphate in the synthesis of polysaccharides, it is felt that the problem of duality of malt amylase is still not settled; at all events the problem is not so simple as most authors now affirm, who believe in the existence of two amylases in addition to amylophosphatase (A. Heiduschka and W. Karsch, *Z. ges. Brauw.*, 1928, 51, 145; E. Waldschmidt-Leitz and K. Meyer, *Z. physiol. Chem.*, 1935, 168, 236). The existence of phosphatases has been proved in malt infusion (H. Lüers and L. Malsch, *Woch. Brau.*, 1929, 46, 143).

Belief in the existence of α - and β -amylases has resulted chiefly from the work of E. Ohlsson (*Hoppe-Seyl. Z.*, 1930, 189, 17) who, as a result of mutarotation experiments found α -maltose to be formed in hydrolysis by the diastase of malt and β -maltose when barley infusion was used. He called the first enzyme "dextrinogenamylase" and the second "saccharogenamylase." Later authors, referring to the convention of R. Kühn (*Liebigs Ann.*, 1925, 443, 1), have called the malt enzyme α -amylase and that of barley β -amylase. However, Kühn—like

Brown and Morris—never found a greater rotatory power than that appropriate to the $\alpha \rightleftharpoons \beta$ maltose equilibrium when maltose was formed by malt diastase action, but always a smaller one. Kühn only found formation of α -maltose by the action of the amylases of pancreas and of *Aspergillus oryzae*. Therefore, there is no α -amylase in malt. The duality of diastase was studied by, *inter alia*, K. Myrbäck (*Proc. Eur. Brewery Conv.*, 1947, 63) by reference to starch constitution, but reservations concerning the hypothesis adopted have already been made above. Recently, R. Sutra (*Bull. Soc. Chim. biol.*, 1948, 30, 439) has confirmed Kühn's work, and states that the principle of duality of malt amylase must not be considered indisputable for α -amylase is both liquefying and saccharifying and β -amylase is saccharifying. However, β -amylase is the enzyme of ungerminated barley—even of ungerminated wheat, infusions of which are both liquefying and saccharifying.

There appears to be some confusion between α -amylase (dextrinogenamylase) and liquefying power and also between β -amylase (saccharogenamylase) and saccharifying power. In view of the possibility of the existence of a liquefying enzyme (in barley or malt) which decomposes phosphoric complexes, we are led to a consideration of the action of phosphatase and amylophosphatase (possibly even phytase) in the hydrolysis of starch and eventually to relationships between the action of such phosphatases and that of the amylase (or amylases) of barley and malt infusions. Thus, potato starch has been hydrolyzed with infusions of barley and malt, with amylases prepared by Ohlsson's method, and with dialyzed amylases. The results were obtained:—

(a) *Hydrolysis by infusions of barley and malts.*— p_H optimum for saccharification 4.7; for liquefaction 6.0. The method of Blom was used (J. Blom, A. Bak and B. Braae, *Hoppe-Seyl. Z.*, 1937, 250, 104) to determine whether the reaction was given by a single enzyme showing a constant ratio of liquefying/saccharifying power at different temperatures for constant p_H and at different values of p_H for constant temperature. However, the ratio is not constant for infusions of barley and malt. It should be noted that liquefying power is difficult to determine, especially in barley infusions

where the action is slight; it was determined by the method of Effront, slightly modified (le Corvaisier, *Thèse Science, Paris*, 1939).

(b) *Hydrolysis by α -amylase.*—With α -amylases of barley and malt prepared by Ohlsson's method but with the modification that heating was carried out in presence of calcium ions at p_H 6, the ratio of liquefying/saccharifying power is less variable. Liquefying power of α -amylases of barley and malt after dialysis is always less than the saccharifying power; in fact, such treatment causes almost the entire disappearance of liquefying power, whilst saccharifying power is diminished by only 50 per cent. Hence, α -amylase is a complex. With α -amylase hydrolyses between the temperature limits 70–82° C. (158–180° F.), the actions of saccharification and liquefaction become more closely comparable; the optimum p_H values are similar but, even so, α -amylase is essentially a liquefying complex. This agrees with the early conclusions of Ohlsson (*C. R. Soc. biol., Paris*, 1922, 87, 1183), that the diastatic complex of malt comprises a liquefying and a saccharifying enzyme, the individual actions of which are difficult to delimit and measure. The difficulty lies in the fact that two distinct activities are present. The simplest explanation would be that each is the result of an individual enzyme, but to resolve the problem it is necessary to elucidate the action of phosphatase.

It must be added that liquefaction of starch by malt amylase affects both the mode and rate of saccharification. Soluble or partly liquefied paste gives more maltose than potato starch paste, when conditions are otherwise the same. Further, the differences between the behaviours of soluble and potato starches diminish as the temperature rises; earlier authors have considered the difference to be due to viscosity, but in experiments made in presence of glycerin it can be shown that viscosity causes, in fact, very little retardation. Therefore it is difficult to deny totally the influence of liquefaction on saccharification, or the influence of α -amylase on the activity of β -amylase; when these enzymes are prepared, it is impossible to destroy the one without impairing the activity of the other. According to E. Kneen, R. M. Sandstedt and C. Hollenbeck (*Cereal Chem.*, 1943, 20, 399; see also F. R. Graesser and P. J. Dax, *Wallerstein Lab. Commun.*, 1946, 9, 43), no

more than 90 *per cent.* of the α -amylase survives when β is destroyed by heating at 70° C. (158° F.) in presence of calcium; more recently Bernfeld (*loc. cit.*) has shown that α -amylase retains 5 *per cent.* of β . Hence separate and accurate determinations are impossible. In fact, α -amylase should only be determined by liquefaction methods, and the difficulty of this has already been pointed out. Saccharification methods may be used for β -amylase. Dextrinization methods involving loss of iodine colour should probably never be used, as the colorations are too uncertain and vary with the colloidal state of the substrate.

It will be of interest to consider the contrasting methods used by various authors for amylase determination. By comparing liquefaction and saccharification curves, some workers have thought that parallelism exists between the two activities (C. Sobolowska and R. Weidlich, *Woch. Brau.*, 1930, 47, 17). On the other hand, Hollenbeck and M. J. Blish (*Cereal Chem.*, 1941, 18, 754) found parallelism between liquefaction and dextrinization—a more understandable result, since the modes of dextrinization and liquefaction must be very similar. Bernfeld (*loc. cit.*) has shown that dextrinization by α -amylase measured by iodine coloration merely shows the decrease in molecular weight of the paste micelles—the result of liquefaction. However, α -amylase action is not confined to liquefaction, and Kneen *et al.* (*loc. cit.*) using saccharification methods and simple conversion formulae found correlation with the results of W. J. Olson, R. Evans and A. D. Dickson (*Cereal Chem.*, 1944, 21, 533) who used dextrinization. S. Redfern (*Cereal Chem.*, 1947, 24, 259) used Baker and Hulton's α -amylodextrin as substrate, as had been proposed earlier by L. Fletcher and J. B. Westwood (*this Journ.*, 1931, 470), who however intended to determine the total activity of barley or malt. The importance of the nature of the substrate is evident.

From the work of H. Lundin (*Woch. Brau.*, 1938, 55, 241) followed by that of E. Sandegren (*Proc. Eur. Brewery Conv.*, 1947, 28) it is known that the proteins of malt, which carry the apoenzyme responsible for amylolytic activity, differ from those of barley in sedimentation characters and micellar weight; also the proteins of wheat differ from those of barley. Therefore, it is

impossible to agree that β -amylase can be isolated from infusion of wheat meal. Action on the constituents of malt or barley infusion is essential to the passage from liquefying to saccharifying activity in the enzyme complex. Experiment shows that, using barley infusion as source of α -amylase, the saccharifying power is inferior to that obtained with malt infusion, for barley infusion has substantially saccharifying power only.

C. E. Danielson and Sandegren (*Acta chim. scand.*, 1948, 1, 917) found that in malt infusion α -amylase (or, better, liquefying amylase) is carried by protein materials which precipitate, in the cold, in the form of small micelles; it is known that the saccharifying amylase (especially of barley) is carried by the larger micelles which precipitate first at high temperature. It should be added that the problem of diastatic activity is further complicated by the possible existence of a factor capable of hindering amylase activity—especially of barley; this factor has been called sisto-amylase, though its exact function is unexplained. Again, impure diastatic preparations have been obtained which are as active as crystalline materials (Meyer, K. Fischer and Bernfeld, *Experimentia, Basel*, 1947, 3, 107).

According to the principles of enzymology, activity depends both on apoenzyme and on coenzyme, these determining specificity. In the present case the coenzyme is unknown; possibly kations play a part. Till evidence is produced to the contrary, it may be imagined that the activity of malt amylase is the result of physical factors involving the micellar size of the substrate and also of the apoenzyme. If barley infusion is kept in a sterile condition for 24 hours it becomes more active than the original infusion (*cf.* J. S. Ford and J. M. Guthrie, *this Journ.*, 1908, 61); during this time there has been partial hydrolysis of protein materials. The same hypothesis allows explanation of the influence of p_H in the inactivation of enzymes; also, the carrier of the liquefying amylase (having smaller micelles) is dialysed before the carrier for saccharifying activity. Molecular size is also important in the substrate; barley amylase more readily attacks degraded starch molecules, and the enzyme attacking the dextrans is only destroyed at approximately 73–74° C. (163–165° F.).

If starch paste is regarded as containing more or less complex colloidal aggregates, hydrolysis may be conceived as the breaking up of the aggregates. Hydrolysis can only take place when the colloidal enzyme-carrier comes into contact with the aggregates, and this requires that the substrate molecules shall be of a particular size. The more complex aggregates give some viscosity to the paste and will be attacked only by malt amylase, having the smaller micelles. The smaller starch aggregates are attacked by the larger enzymic aggregates—by the enzyme of barley, giving maltose directly. This would explain why barley infusion acts more readily on degraded starch than on entire starch. If on the other hand it is admitted that starch paste contains the

A and B fractions of Schoch, it would appear that their relative proportions vary according to the type of starch used; it seems that the proportion of B is smaller than is generally considered, and this would be the fraction responsible for limit dextrin production. It is probable that the variety of dextrans obtained by Myrbäck owed their origin to unduly prolonged hydrolysis, this involving the phenomenon of retrogradation (Bernfeld and G. Gurtler, *Helv. chim. Acta*, 1948, 31, 106).

The Laboratory,
Brasserie Graff Frères,
Rennes.
March, 1949.

MONO-, DI- AND TRISACCHARIDES IN WORT. INFLUENCE OF THE TEMPERATURE OF MASHING

By J. BLOM and B. SCHWARZ

Glucose, maltose and trisaccharides are formed during the mashing process. Changes of the mashing temperature in the range from 60° C. to 75° C. have no measurable influence on their relative amounts. It seems impossible, by changing the mashing temperature, to enrich a wort with trisaccharides in order to influence the secondary fermentation.

IN a previous paper "Trisaccharides in Wort and Beer"¹ we have shown that the fermentable carbohydrates of wort consist of glucose, maltose and trisaccharides. In order to investigate the influence of the temperature of mashing on the relative amounts of mono-, di- and trisaccharides in wort, we have carried out mashing experiments with the same malt at four different mashing temperatures, *viz.* 60, 65, 70 and 75° C. (140, 149, 158 and 167° F.).

The analytical data of the malt were:—

Kenia barley, harvest 1947.

Malted in Saladin boxes for 8 days.

Temperature at dropping from upper to lower floor = 93° C. (199° F.).

Extract, percentage of dry malt = 78.5.

Total nitrogen, percentage of dry malt = 1.73.

Diastatic activity, Lintner value = 37.

The experiments have been carried out as follows: 1 kgm. of coarsely-ground malt is mixed with 5 litres of distilled water at 45° C. (113° F.) in a vessel provided with a stirrer. After maintaining at 45° C. for 15 min. the temperature is raised 3° C. (5.4° F.) *per* minute until the temperatures of 75, 70 and 65° C. respectively are reached. In the experiment where it is desired to keep the mash at 60° C., the temperature is primarily raised to 63° C. (145.4° F.) for about 3 min. in order to gelatinize the starch and it is then lowered to 60° C. After holding the mash at 75, 70, 65 or 60° C. for 90 min. the mash is boiled for 15 min. The entire contents of the vessel are then poured on a sieve and the wort returned until the filtrate is bright.

2 litres of the wort are heated in a steam bath for 2 hours. After 16 hours at room temperature the precipitate is filtered off

TABLE I
DIFFERENTIAL FERMENTATION; CONSTANTS OF FERMENTED PRODUCTS

Experiment.		Mashing temper- ature.	Organism.	Fermen- tation in days.	Per cent. of original extract fermented	$[\alpha]_D^{20^\circ}$	RM	$\frac{[\alpha]_D^{20^\circ}}{RM}$	Mean values		
Group.	No.								$[\alpha]_D^{20^\circ}$	RM	$\frac{[\alpha]_D^{20^\circ}}{RM}$
I	47	60°	<i>Z. marxianus</i>	18	13	20°	132	0.15	24°	128	0.19
	46	65°			11	24°	123	0.20			
	44	70°			9	29°	138	0.21			
	45	75°			9	24°	119	0.21			
II	47	60°	<i>Z. marxianus</i>	24	49	136°	104	1.30	136°	103	1.32
	46	65°	minus		51	136°	104	1.30			
	44	70°	<i>S. uvarum</i>		46	135°	102	1.33			
	45	75°	41		137°	102	1.34				
III	47	60°	<i>S. uvarum</i>	30	9	167°	68	2.47	168°	70	2.39
	46	65°	minus		9	169°	72	2.34			
	44	70°	<i>S. carlsbergensis</i>		9	168°	71	2.35			
	45	75°	8		166°	69	2.40				
IV	47	60°	<i>S. carlsbergensis</i>	2	9	156°	67	2.35	162°	72	2.26
	46	65°	after	2	9	159°	79	2.25			
	44	70°	<i>S. uvarum</i>	2	9	163°	74	2.20			
	45	75°	2	8	171°	77	2.23				

TABLE II
COMPARISON OF LABORATORY AND BREW-HOUSE WORTS
(Results expressed as per cent. of original extract)

Group	Sugar.	Laboratory wort.				Brew-house wort.		
		Experiment No.				Experiment No.		
		47	46	44	45	37	39	42
		Mashing temperature, ° C.						
		60	65	70	75			
I	Glucose	13	11	9	9	13	11	11
II	Maltose	49	51	46	41	43	44	44
III, IV	Trisaccharides ..	9	9	9	8	10	11	12
—	Unfermentable ..	29	29	36	42	34	34	33

TABLE III
COMPARISON OF LABORATORY AND BREW-HOUSE WORTS
(Results expressed as per cent. of fermentable extract)

Sugar.	Laboratory wort.				Brew-house wort.		
	Experiment No.				Experiment No.		
	47	46	44	45	37	39	42
	Mashing temperature, ° C.						
	60	65	70	75			
Glucose	18	15	15	15	20	16	17
Maltose	69	72	71	71	65	68	65
Trisaccharides ..	13	13	14	14	15	16	18

and the filtrate diluted to an extract of 10 *per cent.* The diluted wort is distributed in portions of 150.0 grms. in 300 ml. flasks and sterilized in flowing steam on three consecutive days. The flasks are inoculated with *Zygosaccharomyces marxianus*, *Saccharomyces uvarum* or *S. carlsbergensis* respectively and incubated at 28° C. (82° F.) for about 3 weeks, when the fermentation has come to an end. Dry matter, rotatory power and reducing power are determined before and after fermentation. The methods of analysis are exactly the same as those described in our previous paper, where the calculation of the results and the explanation of the constants $[\alpha]_D^{20}$, RM and $\frac{[\alpha]_D^{20}}{RM}$ are so carefully described that it seems unnecessary to repeat them.

DISCUSSION

The physical constants of the carbohydrates fermented by the three different organisms—group I, II, III, IV—are practically the same in our present experiments (Table I) with laboratory wort as in our earlier experiments with wort from the brew-house. A discussion of the numerical value of the constants seems superfluous, as this is done at length in our previous paper.

The amount of glucose, maltose, trisaccharides and unfermentable matter, found in the present experiment with laboratory wort, are enumerated in Table II together with the amounts earlier found for wort from the brew-house. The results are calculated as *per cent.* of original extract. The amount of glucose, maltose and trisaccharides decreases, and the amount of unfermentable matter increases with increasing temperature. This may be explained by the increasing velocity of inactivation of the amylases with increasing temperature.

Ling and Baker² were the first to report the formation of glucose during the degradation of starch by malt diastases. The maximum amount of glucose formed in

their experiments never exceeded 12 *per cent.* of the total hydrolytic products. It is worth while to notice that the amount of glucose found by Ling³ is in close agreement with the amount of glucose found by us in wort prepared in the brew-house and in the laboratory under normal mashing conditions (60–65° C).

The amounts of glucose, maltose and trisaccharides calculated as *per cent.* of fermentable extract are shown in Table III. The relative amounts of the three carbohydrates are practically independent of the mashing temperature and are of the same order of magnitude in laboratory-wort as in wort from the brew-house. This is remarkable since the relation α -amylase/ β -amylase must alter considerably with the change in temperature, β -amylase being much more unstable than α -amylase at temperatures approaching 70° C.

It may seem hazardous to attempt to explain the constant relation between mono-, di- and trisaccharides by structural arrangements in the malt starch molecule, but the evidence appears to point in that direction.

In our previous paper we have shown that it mainly must be the trisaccharides which give rise to the slow secondary fermentation. In some cases it might be desirable to have a more active secondary fermentation. It seems impossible to meet this wish by changing the temperature of mashing.

We wish to express our best thanks to the management of the Tuborg Breweries.

REFERENCES

1. Blom, J., and Schwarz, B., this *Journ.*, 1947, 302.
2. Ling, A. R., and Baker, J. L., *J. chem. Soc.*, 1895, 67, 739.
3. Ling, A. R., *A Comprehensive Survey of Starch Chemistry*, Edited by R. P. Walton. New York (1928), page 27.

The Laboratory of the Tuborg Breweries,
Copenhagen.

March, 1949.

ABSTRACTS

I.—MATERIALS AND ANALYSIS

Rapid Method for Determining Germinative Capacity in Seeds. H. BRÜCKER (*Physiol. Plantarum*, 1948, 1, 343–358). Many biochemical tests of germinative capacity have been based on the presence of dehydrogenases in living seeds. A completely satisfactory staining test of germinability has not yet been devised, though that of Lakon using tetrazolium salts has proved to be fairly accurate (*cf.* this *Journ.*, 1948, 334). Earlier tests have suffered from transient staining (reduction of dinitrobenzene to nitrophenyl hydroxylamine), presence of toxic material (biselenite test), or unduly high results (reduction of methylene blue). A new test has been evolved, dependent on the activity of peroxidase. Living seeds of the *Gramineae* are rich in this enzyme, and as germinative capacity is lost, peroxidase activity rapidly diminishes. In testing barley, 100 corns are soaked overnight, halved through the embryo, and placed in 20 *per cent.* hydrogen peroxide for 15–30 min. They are then washed in several changes of ethyl alcohol and transferred to a buffer solution of p_H 8.4 for 5 min. Finally the seeds are treated with a mixture of 2 parts of a 10 *per cent.* solution of a saturated alcoholic solution of benzidine and 1 part of a solution of guaiacum. Viable embryos develop an intense violet-red colour which eventually extends over the whole corn. Non-viable seeds are unstained or are stained in less than half the embryo. Instead of bisecting the corns, the embryos may be removed by pressure; this shortens the time required for the performance of the test. The test has shown good agreement with standard germination tests when used with old barley or heated barley, but barley which had been stored for several days under water and which had lost the ability to germinate still gave a strong positive reaction, presumably as a result of bacterial activity.

I. A. P.

Formation of Bitter Acids During Maturation of Hops. M. VERZELE and P. EUGENE (*Fermentatio*, 1948, Nos. 10–12, 95–104). Determination of the α - and β -acids (resins) in hops have been made by a polarimetric method (*ibid.*, 1948, Nos. 4–6) on plants of Kent, Hallertau, Saaz and Tettnang varieties grown at the Belgian National Hop Institute in 1948. Samples were taken from the upper portions of the plants at intervals between 24th August and 24th September. The results are in agreement with those obtained by previous investigators, and show that most of the α -acid is formed during the last days of August and the

early part of September, that the condition of maximum bitter acid content extends over 2 to 3 weeks, and that there is a tendency to start picking too early. Analysis of samples taken from top and bottom of the plants showed that more α -acid was produced at the top and more β -acid at the bottom, and that the total of bitter acids was slightly greater at the top than at the bottom. This was not the case in plants situated on the southern boundary of a garden. On the average, the α -acid amounted to 40 to 50 *per cent.* of the total bitter acids in 1948, which had a dull summer, while corresponding figures on the hop crop for 1947, which had a sunny summer, were 60 to 70 *per cent.* From all these observations it is inferred that there is a close genetic relationship between the α - and β -acids, that humulon is formed at the expense of lupulon, and that this transformation is favoured by sunlight. From experiments made on the drying of hops it is concluded that drying can be done without any loss of either α - or β -acid if the temperature is not allowed to exceed 50° C. (122° F.). In many hop kilns far more lupulin is lost by handling the hops immediately after drying, before they have regained a certain amount of moisture and whilst the cones are fully open. The method used by the authors was to dry the hops in trays on the kiln, afterwards removing the tray but not disturbing the hops until they had cooled and gained a small amount of moisture.

A. A. D. C.

Loss of Bitter Substances During Brewing. P. KOLBACH (*Schweiz. Brau.-Runds.*, 1948, 59, 71–74; through *Chim. et Industr.*, 1949, 61, 378). Experiments have shown that a substantial proportion of the bitter components of hops are removed from wort and beer by the coagulum, the yeast and the subsequent precipitate. These last bitter substances are of value, although they lack the capacity to give any aromatic character to beer, because they include none of the hop oil and the author reviews the methods which might be used to retrieve the loss. Admixture with fresh hops or extraction of the bitter substances with water, with solutions of salts or with organic solvents is suggested, and the most promising results appear to have been given by extraction with a non-decarbonated water.

A. A. D. C.

Hop Diseases in the United States. G. R. HOERNER (*Brewers Digest*, 1949, No. 4, 45–51). A discussion of the more common and more serious, or potentially serious, hop diseases in the U.S., with reference to hop

diseases in Great Britain as reported by Keyworth (*Wallerstein Lab. Commun.*, 1945, 8 99). Crown gall (*Agrobacterium tumefaciens*) has been found for many years in California, Oregon and Washington, but is not mentioned by Keyworth. There are no well-defined symptoms except the presence of the galls, and no appreciable damage is done to the plant unless the entire crown becomes diseased, but much damage and loss can be caused to nursery stock. The bacteria persist in the soil for a considerable time, and may be carried mechanically (on tools, in irrigation water, etc.) and introduced through pruning cuts, or spread by planting infected cuttings. All commercial varieties are susceptible, and badly infected plants should be completely removed and the underground portions burnt. Downy mildew (*Pseudoperonospora humuli*) is the most serious of hop diseases in the U.S. Counter-measures used are treatment of the crown with calcium cyanamide, prompt removal and destruction of basal, terminal, lateral and nodal spikes as they appear, overhead sprinkling, application of liquid or dust fungicides as soon as the vines have been strung, and progressive stripping of the lower leaves. Spreaders are desirable with liquid sprays, which have been largely abandoned in favour of dusting on the Pacific Coast. All commercial varieties are susceptible, but some less so than others. Sooty mould (*Cladosporium aphides*) causes serious losses every year. The fungus grows on honeydew excreted by aphides, discolours the leaves and enters the cones. Control consists in preventing the preliminary aphid infestation. Powdery mildew (*Sphaerotheca humuli*) is confined to New York, where it is sometimes called "blue mould." Control is by dusting or spraying with sulphur at the first signs of the white patches on the leaves, and the number of applications will depend on the weather and the severity of the early infection. Early training and stripping of the lower leaves will assist control. Root rots (so-called) are causing increasing concern to growers on the Pacific Coast, especially in Oregon. They are usually associated with mechanical injuries to the crowns, are generally dry, accompanied by brown or black discoloration of the tissue, and cause loss of newly planted cuttings and gradual dying of established plants. There is widespread rotting of the underground stolons, even from crowns which appear to be healthy. No direct control measures can be suggested, but care in pruning and cultivation to avoid undue injury will be helpful, and only healthy cuttings should be used. Rot due to *Armillaria mellea* is of minor importance in the U.S., and the rot due to *Phytophthora cactorum* has not been definitely identified there. Seven disorders are suspected of being induced by virus infections: *Chlorosis*,

Leaf distortion, *Ring-spot* and *Slip-down* affect "English" Clusters, *Yellow Flecking* is common on "English" Clusters, *Brewer's Gold* and other varieties, *Split-leaf Blotch* affects Fuggles, and the seventh is *Ring-spot Chlorosis*. *Slip-down* has symptoms similar to those described by Keyworth for Fluffy Tip and Nettlehead, *Split-leaf Blotch* appears to be the same as the disease of that name described by Keyworth, and the symptoms of *Leaf distortion* are very similar. The other four are not mentioned by Keyworth. The commonest virus disease, Mosaic, commonly called "blight" in Oregon, has caused as much as 40 per cent. reduction in yield in a single season and entire plantings have been killed in a few years. Means of control of these virus diseases are incomplete. Affected plants should be removed promptly, cuttings should not be taken from diseased varieties, and varieties known to be carriers should be kept away from susceptible varieties. *Verticillium* wilt has not been definitely identified in the U.S.; *Canker* (*Fusarium* sp.) as described by Keyworth has been observed only in one area and, although probably widespread, appears to be of minor importance. The paper is well illustrated by photographs. A. A. D. C.

Fluorimetric Estimation of Nicotinamide in Biological Materials. D. K. CHAUDHURI and E. KODICEK (*Biochem. J.*, 1949, 44, 343-348). A fluorescent compound can be obtained by the action of cyanogen bromide on nicotinamide without recourse to an organic solvent, by use of acid and alkaline pretreatments of samples and a special blank to eliminate interference from other fluorescent substances, e.g., aneurin and coenzyme I. The nicotinamide content of a sample is determined by comparison with a sample to which known amounts of nicotinamide have been added. This internal standard is adopted in order to eliminate the quenching effect of nicotinic acid on the fluorescence developed. Readings are made in a "one photocell type" fluorimeter. The procedure determines both free and bound nicotinamide. In baker's, brewer's and dried yeast and in wheat germ, roughly one-half of the vitamin was present as nicotinamide. In contrast, all, or nearly all the vitamin of wheat bran, 100 per cent. extraction wheat flour and marmite (autolysed yeast) was present as nicotinic acid. C. R.

Fluorimetric Estimation of Riboflavin. E. KODICEK and Y. L. WANG (*Biochem. J.*, 1949, 44, 340-343). The method estimates the vitamin directly, without recourse to lengthy adsorption procedures. Samples, containing about 15 µgrm. of riboflavin, are extracted on the water bath with 0.1 N hydrochloric acid. Proteins are precipitated with metaphosphoric

acid and, in the case of starchy materials, a digestion with takadiastase is given. Interference by unspecific fluorescent substances is reduced by washing with chloroform, followed by oxidation with permanganate, excess of which is removed by hydrogen peroxide. The almost colourless extract is adjusted to p_H 6 and readings made in a Cohn-type single photocell fluorimeter using blue light. Comparison is made with a standard solution in 20 per cent. alcohol containing 5 μ gm. per ml. of riboflavin. For blank determinations, the riboflavin in the sample is reduced by a neutral solution of sodium dithionite to a non-fluorescent compound. C. R.

Assay of Vitamin B₆ with *Neurospora sitophila* M.299. S. MORRIS, G. HERWIG and A. JONES (*Analyst*, 1949, **74**, 37-43). The organism is maintained as resting spores in 10 per cent. sucrose and transferred to malt extract-peptone agar at 30° C. three days before an inoculum is required. Under these conditions, the mould retains its activity for the assay over long periods. The assay medium consists of sucrose, ammonium tartrate, sodium dihydrogen citrate, inorganic salts and biotin. The presence of aneurin interferes with the assay. Aneurin in test samples must therefore be destroyed prior to assay. Treatment with sodium sulphite and hydrogen peroxide is not suitable for this purpose, the procedure chosen involving heating for 1 hr. at 15 lb. pressure with N sodium hydroxide. The samples are then adjusted to p_H 4.6 and diluted to give approximately 0.3 μ g. of vitamin B₆ per ml. Ultra-violet light may totally inactivate vitamin B₆ but ordinary artificial light has little effect. The response of the mould in the assays is measured by weighing the mycelium. C. R.

Assay of Folic Acid with *Streptococcus faecalis* NCTC 6459. A. JONES and S. MORRIS (*Analyst*, 1949, **74**, 29-36). The medium resembles those used for the microbiological assays of members of the vitamin B complex (this *Journ.*, 1945, 100), but includes charcoal-treated peptone and also sodium citrate as buffer. The organism, maintained as stabs on yeast extract-glucose-sodium acetate agar, loses its power of response to folic acid within a 16-hr. incubation period. Consequently, transfer was made, through liver-tryptone broth, to liver-tryptone agar slopes. With inocula prepared from these slopes, response could be measured by titration with 0.1 N sodium hydroxide, or turbidimetrically, after 16-18 hr. incubation. Extraction with takadiastase resulted in "drift" in the assay results of test samples and loss in the case of standard folic acid. Autoclaving with 0.1 N HCl or 1 per cent. sodium acetate gave higher figures for folic acid

from yeast, possibly due to the splitting of folic acid conjugate, but with pure folic acid the acid treatment resulted in loss. No method for the assay of free folic acid could be found, so that the assays carried out gave figures for total (free and conjugated) folic acid. The total folic acid was liberated from test materials by treatment with a crude enzyme preparation from hog kidney. Values obtained with *S. faecalis* agreed closely with those obtained with *Lactobacillus casei*, but the former was preferred as test organism. Some loss of folic acid occurs on exposure to ultra-violet light, but ordinary artificial light has little or no effect. C. R.

Evaluation of Diastatic Activity. G. I. DE BECZE, J. W. VOTAW, jun., and R. H. NANZ, jun. (*Cereal Chem.*, 1949, **26**, 148-159). Several methods have been used for determining amylase activity, and the authors have modified the Lasché procedure in order to render it applicable to materials of widely varying diastatic power. The results are expressed as converting capacity, which is defined as the number of grms. of soluble starch converted to the iodine-achromic point by 100 c.c. of the test solution in 1 min. at 62.5° C. and p_H 4.0-5.2. After investigating the effects of the various factors involved the authors devised the following method: The material to be tested requires no preparation if a liquid, but a paste or syrup should be diluted and solid materials must be pulverized and extracted with water, any undissolved matter being removed by sedimentation, centrifuging or filtration. A fresh solution of soluble starch containing from 1 to 5 gm. of the solid (on a dry basis) is placed in a beaker with sufficient water to yield a total volume of 100 c.c. when the solution under test is added. After warming in a water-bath to 62.5° C. the amylase solution at the same temperature is run in and after digestion has progressed for 3 min. 2 drops are removed, mixed with the same volume of 15 per cent. acetic acid on a spot plate and 2 drops of 0.001 N iodine are added. Successive tests are made at intervals of 30 sec. until the achromic point is approached, when the time between taking samples is reduced to 15 sec. The conversion time is the number of minutes elapsing between addition of the enzyme solution and that at which the achromic point is reached. The converting capacity is calculated from the formula $100S/L.t$, where S is the weight of starch in the digest, L the volume of the diastatic solution added and t the conversion time. The digest is allowed to cool and its p_H is then determined. The quantities of starch and enzyme solution required may be roughly estimated from the nature of the test material and its expected converting capacity. The method has been successfully used for

determining the amylase activity of malt and samples from any stage of distillery procedure.
T. J. W.

Chromatographic Separations. H. H. STRAIN (*Analyt. Chem.*, 1949, **21**, 75-81). A review of the subject, containing 152 references to the literature. In a section on *Methods* the author discusses types of adsorption tubes and devices for removal of the adsorbent and examination of the percolate fractions, and briefly describes the procedures and aims of column and paper chromatography. Study of the distance migrated by the solute in relation to that migrated by the solvent (frontal analysis), development of the chromatogram until the solutes are carried into the percolate (elution analysis), and development with a series of solvents or solvent mixtures of increasing polarity to the same end (displacement development), are examples of columnar procedure; and the system of two-dimensional development, in which the chromatogram is developed further in a direction at right angles to that of the first development, is a procedure possible only in paper chromatography. Such methods can be used not only for resolving mixtures and isolating components but also for comparing, describing (in terms of chromatographic behaviour), identifying and purifying substances. A section on *Adsorbents* contains references to ion exchangers (for separation of substances of different ionic charge), partition chromatography (in which a solvent, held in some material such as silica gel or Kieselguhr, behaves as an adsorbent), and surface-active adsorbents, and discusses the selection of adsorbents. There follow sections on *Solvents and Eluants* and *Applications*, and in a final section on *Principles of Chromatography* there are references to the mechanism, rate of migration of solutes, and mathematical interpretations of chromatographic separations. A representative table of substances which have been separated or isolated by adsorption, and the adsorbents and solvents used, is included in the review.
A. A. D. C.

Filter Paper Chromatography. H. B. BULL, J. W. HAHN and V. H. BAPTIST (*J. Amer. chem. Soc.*, 1949, **71**, 550-552). This paper describes an attempt to make the filter paper chromatography method of Consden, Gordon and Martin (this *Journ.*, 1945, 48) for amino acids quantitative. The procedure devised is, in essentials, as follows. Several strips of Schleicher and Schüll quantitative filter paper No. 507, 7.2 mm. wide and 60 cm. long and cut against the machine direction, have one end held in the bottom of an oblong dish, the strips hanging over the sides being weighted at the other end. On a marked spot on each

strip, about 4 cm. from the edge of the dish, is placed 0.0135 c.c. of the amino acid solution, adjusted to a p_H of 5 to 7, the strip being held horizontally for the spotting. The strips are allowed to dry, and the dish and strips then placed on a stand in a tall glass jar containing a layer of 80 per cent. aqueous phenol solution on the bottom. Sufficient of the same phenol solution to cover the ends of the strips is added to the dish, a second glass jar inverted over the first, and the joint between the two jars sealed with vaseline. After about 48 hours, the phenol solution having nearly reached the ends of the strips, they are removed, the flow distance of the phenol marked with a pencil, and the strips allowed to dry in the air. They are then heated for 2 min. at 90° C. (194° F.) in an oven with a circulating fan, removed and stretched horizontally, and sprayed with a freshly-prepared solution containing 0.40 gm. ninhydrin, 10 gm. phenol and 90 gm. *n*-butyl alcohol. After drying out, the strips are sprayed on the reverse side and allowed to dry again, placed in an oven at 90° C. for 10 min., and finally over an enclosed steam-bath for 5 min. to develop maximum colour. The strips are mounted in a groove 7.2 mm. wide on a glass slide with the glass on either side blacked out, and passed between the illuminated slit and the phototube of an electronic photometer. The slit is 6 mm. high and 5 mm. wide, and a light filter with maximum transmission at 570 m μ . is placed in front of it. The light transmission is read every 5 mm. along the strip, and expressed as per cent. of the light transmitted through a control strip treated in the same way but omitting the amino acid. These values are plotted against distances along the strip on semi-log graph paper, and the area above the plotted curve and 100 per cent. transmission is measured with a planimeter. This area is multiplied by the standard colour-area of the particular amino acid to obtain the concentration of the acid, which is identified by its R_F value as defined by Consden *et al.* (*loc. cit.*). In case of doubt, known amino acids may be added to the amino acid mixture and the position of the increase in areas of the light transmission/distance plot noted. The authors discuss the experimental data which determined this procedure, and tabulate the R_F values of a number of amino acids as a function of phenol concentration and as a function of the p_H of the amino acid solution. Contrary to some reports, the tint of the colour developed with ninhydrin was the same for all the amino acids investigated, provided the p_H of the solution was kept between 4 and 7.5. Using graph paper with a space of 2 mm. between lines, and measuring the areas above the graphs in sq. in., it was found that the points for arginine, serine, valine, glutamic acid, leucine, threonine, alanine and

lysine fell about a line whose least square equation was $A/C = 380.4 - 9.10 A$, where A is the area and C is the amount of the amino acid in micromoles. The probable *per cent.* error for 68 determinations extending from 0.67 to 13.5 micromoles of these acids was ± 8.82 . Four other amino acids developed less colour *per* micromole, and the equations for these are: aspartic, $A/C = 340 - 10.0 A$; phenylalanine, $A/C = 210 - 6.5 A$; methionine, $A/C = 160 - 0.5 A$; glycine, $A/C = 275 - 12.3 A$. An example of the application of the method to the resolution of a mixture of amino acids is described. All resolutions have been done at room temperature and, using 80 *per cent.* phenol; the only effect of varying the temperature was that the phenol moved faster at higher temperatures. If this movement became too fast separation of the amino acids was not sharp. If temperature gradients exist, the strips should be arranged so that they face the gradient; otherwise the amino acid bands will be distorted. A. A. D. C.

Filter-paper Partition Chromatography of Sugars. S. M. PARTRIDGE (*Biochem. J.*, 1948, 42, 238-248). Partition chromatography on strips of filter paper, previously successfully used for the separation of amino acids (*cf.* this *Journ.*, 1947, 171), has now been shown applicable to the qualitative analysis of sugars in biological fluids and in polysaccharide hydrolyzates. Filter-paper strips (*e.g.*, 43 $\times$ 12 cm.) are suspended in an enclosed vessel in an atmosphere saturated with respect to the vapour of water and of the solvent to be used, the upper end of the strip being bent over to dip into a trough containing the solvent saturated with water. Spots of sugar solutions (which may contain 5-40 microgm. of the sugars) are applied on a marked horizontal line near the top of the paper. The solvent forms a well-defined "front" which crosses this line and travels down the paper, and the sugars move down also at a lesser rate dependent on their own nature and on the nature of the solvent. After an appropriate time (*e.g.*, overnight) the position of the front is marked, the paper rapidly dried, and then sprayed with an ammoniacal solution of silver nitrate. On drying again, the positions attained by the sugar spots are shown by brown stains on the paper. It is found that the position reached by each sugar, relative to the total distance travelled from the starting line by the solvent, is characteristic for that sugar under constant temperature conditions with a given solvent. Thus, if known sugars are run as controls on the same paper with an unknown sugar mixture, the components of the mixture are separated and individuals can be identified by their positions relative to the controls. Solvents

which may be used include phenol saturated with water (which gives good separation of glucose and xylose and of arabinose and galactose, though not of xylose and galactose) and an aqueous mixture of *n*-butanol and acetic acid (which gives good separation of xylose and galactose, but not of glucose and galactose). Electrolytes may interfere with sugar separations in this method, and preliminary removal of salts, acids and bases is advisable, *e.g.*, by utilizing ion-exchange reagents. I. A. P.

Quantitative Analysis of Mixtures of Sugars by the Method of Partition Chromatography. Part I. Standardization of Procedure. A. E. FLOOD, E. L. HIRST and J. K. N. JONES (*J. chem. Soc.*, 1948, 1679-1683). A method is described for the separation and determination of mixtures of sugars of total weight about 0.1 to 1.0 mgrm. on the paper chromatogram. The technique is an elaboration of Partridge's application (preceding abstract) of the paper chromatogram method of Consden, Gordon and Martin (this *Journ.*, 1945, 48), and with experience is capable of accuracies of ± 2 *per cent.* The apparatus is similar to that used by Consden *et al.*, with substitution of a bell jar with ground glass flange for the drain pipe, and the paper is Whatman No. 1 cut to lengths of 60 cm. The solvents and procedure are as described by Partridge. The solution containing the sugars must be neutral and the concentration of inorganic ions kept to a minimum. The concentration with respect to the individual sugars is adjusted to be of the order of 1 *per cent.* Spots of the order of 0.002-0.004 c.c., of uniform size, are placed about 5 mm. apart at the top of the paper, and the chromatogram run for 45 hr. at 20° C. (68° F.) using a mixture of *n*-butyl alcohol (40), ethyl alcohol (10) and water (50). Ammonia is added to the aqueous phase to a concentration of 1 *per cent.* After drying for 1 hr. at 100° C. a strip containing one end-spot is cut from each paper, sprayed with ammoniacal silver nitrate solutions, and returned to the oven for development. By matching this strip alongside the sheet from which it was cut, areas can be cut from the latter each containing one only of the separated sugars; at the same time areas free from sugar and equal in size to each of the areas containing sugar are cut to serve as paper blanks. The sugar is removed from the paper by a process of flushing with 5 c.c. water by reflux for 30 min., and the paper blank treated in the same way. A further 5 c.c. of water is taken as a water blank, and a fourth 5 c.c. of a standard aqueous solution (20-50 mgrm. *per* litre) of the sugar under analysis. The copper-reducing value of each of these four is determined with Somogyi's copper reagent (*J. biol.*

Chem., 1945, 160, 61) and the weight of sugar from the chromatogram calculated by simple proportion. The paper blank is constant, and varies little from paper to paper. If it is not certain that the sugars analyzed are the sole constituents of the mixture being examined, a sugar known to be absent from the mixture and having a R_F value different from those of the sugars present is weighed in before chromatography to serve as a reference compound. Instead of the copper reagent, potassium ferricyanide may be used with the same technique, but the former is preferable. Examples of the analysis of mixtures of sugars are given. Polysaccharides in which the ratio of sugars has been determined by other means have been analyzed by the new method, and the corresponding sets of results are in agreement. Attempts to resolve *dl*-mixtures of sugars by the use of an optically active alcohol such as menthol or amyl alcohol in place of butyl alcohol have not been successful. Full working details are given in the paper.

A. A. D. C.

II.—FERMENTATION

Practical and Inexpensive Method for Preserving Yeast. E. MUELLER (*Amer. Brewer*, Feb., 1949, 82, 54). The following procedure may be carried out without the use of expensive apparatus or a laboratory, and enables the brewer to maintain a stock of yeast which he has proved suitable in his business. Into each of six 2-3-litre Erlenmeyer flasks 50 grms. of sucrose and 500 c.c. of water are placed and after boiling for 15 min. the necks are closed tightly with cotton wool. After cooling the flasks are transferred to a cellar for several days to determine if the media are sterile as shown by their remaining clear. A suitable brewery yeast of the second to fourth generation is washed thoroughly, pressed in a filter press and examined microscopically to ensure that it is free from foreign organisms. After sterilizing his hands with alcohol the operator transfers about 200-300 grms. of the yeast to each of the flasks which are quickly closed and transferred to a room maintained at a temperature of 39-41° F. During the first 2 days slight fermentation may occur, but the yeast will remain viable indefinitely. When a brewery replacement is necessary the contents of one of the flasks are transferred to a small vessel of wort and propagation is continued in increasingly larger vessels until sufficient yeast is obtained to start a full scale brew which may be continued for 2 or 3 months, when the second flask is used in a similar way. After the fifth or sixth flask has been transferred the entire process is repeated with the same or another type of yeast if desired.

T. J. W.

Sporulation in Yeasts. II. H. J. PHAFF and E. M. MRAK (*Wallerstein Lab. Commun.*, 1949, 12, 29-44; cf. this *Journ.*, 1949, 120). It has been known since the 19th century that some of the vegetative cells of yeasts are capable of undergoing sporulation. Of the two principal methods of inducing spore formation, the first consists of transferring actively growing vegetative cells to the sporulation medium (e.g., gypsum). In the second, the yeast actually grows on the sporulation medium (e.g., vegetable wedges or Gorodkova's agar). Knowledge concerning the factors involved in sporulation is very limited but it is recognized that there is no simple answer. Not only do many factors influence sporulation of one yeast but there is no uniformity between species. No generalization is possible and the development of very complex sporulation media has therefore taken place, the object being the incorporation of sufficient factors to cause sporulation in a wide range of species. Attempts have been made to isolate and identify factors responsible for sporulation in certain media and it is known that physical factors and genetic considerations are important. The influence of temperature and loss of ability to sporulate in old cultures are examples of the former. It is advisable to investigate new cultures of yeasts as soon as possible after their original isolation.

All workers agree that abundant oxygen is required for adequate sporulation and generally room temperature has been found most satisfactory, but different species vary in their temperature requirements. Reports on optimum p_H are contradictory but a range of 4-7 appears suitable. Moisture appears to be necessary and of the various sources of carbon, glucose appears to be generally satisfactory; it is used in concentrations of from 0.1 to 0.25 per cent. The source of nitrogen is important as ammonium salts and some amino acids sometimes inhibit sporulation. A complex mixture of high and low molecular weight proteins and their hydrolyzates in quite small quantities is apparently satisfactory. Extracts and broths from other organisms, particularly moulds, have been reported to stimulate sporulation and yeast autolyzate has been observed to have similar properties. For adequate sporulation, young vigorous cultures are required and two successive passages through wort or grape juice are advised. The cultures are then best transferred to vegetable infusion-agar media, vegetable wedges or Gorodkova's agar, whilst film-forming species may be studied by examination of the pellicle on grape juice. To hasten sporulation, cells from a vegetable-agar slant may be transferred, after washing, to a gypsum slant moistened with dilute wort, 0.25 per cent. K_2HPO_4 -mannitol solution or dilute acetic acid at p_H 4.0.

A. E. W.

Gene for Diploidization in Yeasts. O. WINGE and C. ROBERTS (*Compt. rend. Trav. Lab. Carlsberg, Sér. physiol.*, 1949, **24**, 341-346). The American "Yeast Foam" strain of *Saccharomyces cerevisiae* was markedly heterothallic, single spores affording haploid cultures which rarely underwent diploidization. In each four-spored ascus two of the spores belonged to one mating type and two to another (*cf.* Lindegren and Lindegren, *J. Bact.*, 1943, **46**, 405). Reaction between haploid cultures of opposite mating types was studied and in some cases copulation and diploid zygotes were observed. Under favourable conditions the latter developed vegetatively and were capable of forming ascospores. *S. chevalieri* was homothallic; all single spore cultures diploidized.

By placing one spore from each species within the operation chamber, a fertile hybrid was obtained, which exhibited segregation of biochemical characteristics. When single spore cultures were mated with each other an apparent tetrapolar mating scheme resulted; similar results were obtained when single spore cultures from the hybrid were mated with the two *S. cerevisiae* mating types. By indicating the mating types of *S. cerevisiae* by *A* and *a*, and the gene pair responsible for whether or not a mating reaction occurs by *D* and *d*, a table was constructed showing the presence or absence of a mating reaction in back-crossing to the two possible mating types of *S. cerevisiae*. The apparent tetrapolar mating thereby revealed appeared remarkable in the progeny of a hybrid between a species with a dipolar scheme and a homothallic species. In the table, results from experiments with 30 asci are shown. Where two single-spore cultures from the same ascus of the hybrid react with the same mating type the formula of all spores is easily determined. This holds for three asci in which two spores had the formula *AD* and two *ad* and for four asci having two *Ad* and two *aD* spores. However, in the remaining 23 asci all four spores were of a different formula, namely *AD*, *Ad*, *aD* and *ad*. Only *Ad* and *ad* participated in a mating reaction and of the two remaining spores it was impossible to determine which was *AD* and which *aD*. An *ad* type and an *aD* type were then selected from those asci in which the formulae of the spore progeny were known and an attempt was made to cross these with the unidentified spore cultures from the 23 asci. The actual results showed no mating reaction in any case; actually all 60 single-spore cultures from the hybrid which contained *D* were diploid, capable of sporulation and therefore no longer active in mating experiments.

The apparent tetrapolar mating was therefore illusory but the results are explained in another way. Thus, interaction exists between the mating gene pair *A-a* and the gene pair *D-d*

responsible for the presence or absence of diploidization at spore germination. The formulae for the mating types of *S. cerevisiae* were revised to *ad*, *Ad*, *ad* and *Ad*, and the results of the mating were presented in tabular form together with the results of reciprocal crosses with two of the mating types from the F1 hybrid. 720 mating experiments were involved and four unexpected results were obtained for which there has been no satisfactory explanation. It was evident that a simple bipolar mating was involved and that in *S. chevalieri* there occurs a gene, *D*, which controls diploidization of its single-spore cultures. The diploid formula of the species is therefore *DD*. In the strain of *S. cerevisiae* diploidization does not normally occur and the strain therefore lacks gene *D* and may be designated as *dd*. The hybrid will be of formula *Dd* and in its asci two *d* and two *D* will be segregated. Possession of gene *D* involves obligatory diploidization but *d*-spores do not produce only haploid cultures. From the 120 single-spore cultures from the hybrid, 57 of the 60 *D* types sporulated on gypsum. From the 60 *d*-types, 11 produced asci but only four of these showed good spore formation. Mating between the hybrid's single-spore cultures in reciprocal crossing was sometimes less pronounced than between the hybrid's single-spore cultures and *S. cerevisiae* spore cultures, presumably due to mating type being influenced by modifying genes.

A. E. W.

"Direct" Fermentation of Disaccharides by Yeast. A. GOTTSCHALK (*Wallerstein Lab. Commun.*, 1949, **12**, 55-69). The usual method of disaccharide breakdown by micro-organisms involves a preliminary hydrolysis to monosaccharides. In the past decade an alternative mechanism has been demonstrated for some bacteria, namely the phosphorylase system which initiates a direct breakdown without the participation of a molecule of water. It is therefore of interest to re-examine the theory of Willstätter who postulated a "direct" fermentation of disaccharides by yeast. One basis of the theory was that fresh yeast will ferment maltose in p_H 4.5 buffer, but an autolysate of the same yeast exhibits no maltase activity. However, the interior of the living yeast cell has a buffer system maintaining a p_H value of about 6.0 even in media of considerably different p_H . The value of 6.0 is close to the optimum for maltase activity. In the second place, the enzyme system in the yeast all remains in concentrated solution, whereas that in the autolysate is diluted and therefore unstable. More recent support for the theory has been claimed by Leibowitz and Hestrin, who have compared fermentation rates of maltose and methyl- α -glucoside. The latter is presumably hydrolyzed by maltase prior to

fermentation and it was found that, over a range of temperatures and acidities, the alteration in the rate of maltose fermentation was much less than that of methyl- α -glucoside. These facts were presumed to demonstrate a direct fermentation of maltose, but no account was taken of the differential effect of p_H and temperature on the permeability of the cell wall to the two substances. Methyl- α -glucoside penetrates the cell wall of yeast much more slowly than does maltose and this fact would account for the differences in fermentation rates. The variation of permeability of the cell wall towards different sugars has been demonstrated by the difference in fermentation rates *in vivo* against the much more similar rates when these are carried out by cell-free preparations.

Methyl- α -glucoside additions have an inhibitory effect on the fermentation of maltose by baker's yeast and this evidence has also been cited in support of the "direct" theory. However, the facts may be simply explained by the similarity of chemical constitution of the two substances and the different rates of penetration to the cell interior. Similar evidence has been presented for the "direct" fermentations of sucrose and lactose but is considered to be invalid for similar reasons. Brewer's or baker's yeast contains considerable quantities of the non-reducing disaccharide trehalose. It is produced in cells fermenting glucose and fructose. Since the hydrolases catalyse irreversible reactions, the formation of trehalose by a different mechanism may be surmised. Additional support has been obtained by the isolation of trehalose monophosphoric ester, but as no further details are available it is mere conjecture to assume that the same enzyme may be responsible for the fission of the disaccharide.

A. E. W.

Fermentation of Disaccharides. II. Sucrose. S. HESTRIN (*Wallerstein Lab. Commun.*, 1949, **12**, 45-54). The metabolic transformation of sucrose aptly illustrates the diverse modes of cleavage which a disaccharide may undergo. Several sucrose hydrolases are known, of which yeast invertase has been the most studied. Invertase in all preparations hydrolyzes only those saccharides possessing a terminal β -fructofuranoside group, *e.g.*, sucrose, raffinose and stachyose. A second sucrose is produced by yeast and acts at nearly neutral p_H hydrolyzing sucrose, maltose and melezitose but not raffinose; it is thus an α -glucosidase and may be identical with maltase. Several sucrases can be isolated from fungi and bacteria but these differ considerably from yeast sucrases, particularly in respect of optimum p_H . Invertase hydrolyzes sucrose much faster than the components can be

fermented, and an opportunity is thus afforded of studying its action in the living cell. The behaviour is very similar to that found with cell-free preparations. The fructofuranose liberated by hydrolysis is unstable and isomerizes to a mixture of the furanose and pyranose forms with the latter predominating. It is the furanose form which undergoes fermentation, since the free hydroxyl at carbon 6-position is capable of accepting phosphate.

Non-hydrolytic pathways of sucrose metabolism have been demonstrated in many bacteria, and may well occur with yeasts; the invertase activity of yeasts is so great however that other possible methods of breakdown would be obscured. These other methods are exemplified by the sucrose metabolism of *Leuconostoc mesenteroides* which forms a polysaccharide from the glucose half of the molecule, the fructose half being liberated. Neither water nor phosphate takes part in this reaction, and similar specific mechanisms are responsible for the production of other bacterial dextrans and levans. A further enzyme mechanism has been demonstrated in cell-free preparations from *Pseudomonas saccharophila*. The enzyme, a transglycosidase, in presence of phosphate, transforms sucrose to a mixture of glucose-1-phosphate and fructose, and in the absence of phosphate the reverse reaction is catalyzed. The enzyme is specific only in so far as the glucose portion of the molecule is concerned, considerable latitude being permissible with the second group. It is difficult to assess the importance of phosphorolysis as a general method of disaccharide cleavage but the latitude quoted encourages the belief that lactose, maltose and trehalose will prove capable of entering into reactions with phosphate in the presence of suitable enzymes.

A. E. W.

Comparative Studies of *Acetobacter* Species with Particular Reference to Beer Contaminants. T. K. WALKER and D. KULKA (*Wallerstein Lab. Commun.*, 1949, **12**, 7-28). Members of the genus *Acetobacter* are very widely distributed in nature, and in any natural medium such as milk, beer or wine initial infection is by chance. Should suitable nutrients be present, the organism will become established and in due course its behaviour becomes somewhat modified. Acetic acid bacteria occur in most top-fermentation yeasts and their presence may be sought by microscopical examination of yeast suspensions after treatment with 10 *per cent.* caustic soda (to remove confusing protein and resin particles). The Gram-negative *Acetobacter* spp. often may be distinguished from other Gram-negative bacteria as chains of coccoid cells frequently in diplo arrangement. To facilitate isolation from yeast, fractional centrifugation may be adopted:

alternatively, the yeast may be cultivated in sterile beer, when the developing *Acetobacter* will be found above the deposited yeast. From such enriched preparations, pure cultures are isolated in the usual manner. Malt wort with ethyl alcohol and yeast-water with glucose, glycerol or alcohol are useful media for growth, and yeast autolysate or corn steep liquor may be used as alternative sources of nitrogen. In liquid media, *Acetobacter* spp. should be transferred at monthly intervals and should be stored at room temperature. Stock cultures are prepared in liquid media with the addition of light calcium carbonate and after 5 days at the optimum temperature the tubes are sealed and stored in a cool place.

The genus is characterized by ellipsoidal or rod-shaped, motile or non-motile cells. Its members are without spores, are Gram-negative and do not liquefy gelatine. Many organic compounds are oxidized. Species are recognized by the type of growth in wort and beer, and by the appearance of streak cultures and giant colonies. Some typical photographs of the latter are shown. Certain biochemical reactions are also employed for identification purposes. Variation is often encountered in some species and this must be looked for when attempting identifications. Most members of the genus show ketogenic activity but this is not a reliable diagnostic characteristic. *Acetobacter capsulatum* is one of the most widely occurring species in the brewing industry but other species encountered are *A. turbidans*, *A. aceti*, *A. acetosum*, *A. suboxydans*, *A. mobile* and *A. acidum-polymyxa*, and a table is presented giving the appearance and properties of wort, beer and wort-agar cultures of six of these species. All the species enumerated have adverse effects on beer. Capsule formation is a characteristic of the genus and may be demonstrated by Barker's staining technique. Shimwell has noted that *A. viscosum* produces rope in the presence of dextrin and the capsules of the organism have been shown by Kaushal to consist of glucose units and some uronic acid residues. Some species also produce cellulose films. Infection with *Acetobacter* spp. may be avoided by the use of pure yeast, and thorough attention to cleaning. Sulphite is a useful antiseptic and in beer the exclusion of air will minimize the effects of contamination.

A. E. W.

Brewing Bacteriology. VI. Lactic Acid Bacteria (Family *Lactobacteriaceae*). J. L. SHIMWELL (*Wallerstein Lab. Commun.*, 1949, 12, 71-88). Although lactic acid is found amongst the metabolic products of many bacteria, the term "true lactic acid bacteria" is reserved for those whose main product of carbohydrate breakdown is lactic acid. They are Gram-positive, non-motile, non-spore-

forming and require a complex nutrient system. They are a physiological rather than a morphological group and while some are rods and some cocci their physiological characteristics are often very similar. The lactic acid produced may be *dextro*, *laevo*, or a mixture of the two. If lactic acid is the sole end product, the strain is designated "homofermentative," but if a little alcohol, carbon dioxide or other products are formed the term "heterofermentative" is used. On morphological grounds lactic acid bacteria are divided into two genera—*Streptococcus* and *Lactobacillus*. They are very widely distributed and due to the extreme economic importance of the group, classifications have often been adopted according to the habitat being studied. For brewing purposes it is simpler to think only of the two genera mentioned above.

Lactobacillus includes *L. delbrückii* which is thermophilic and homofermentative and is the well known mash souring bacterium; its outstanding characteristics are high optimum temperature, extreme variation in morphology and inability to ferment lactose or grow in beer. *L. leichmanii* is very similar but has a much lower optimum temperature; it is often found as chains of short cells and is unable to grow in beer. *L. plantarum* comprises most mash-tun, lactose-fermenting strains. Of the beer-spoilage strains, *L. pastorianus* causes a silky turbidity and acidity in beer; its morphology varies from short rods (in wort or on wort agar) to long rods (in beer, particularly if of high alcoholic content). On solid media growth is slow, and sugar fermentations vary according to strain. Increasing hop rates progressively inhibit growth but even high rates (4 lb. per barrel) do not entirely prevent it. The spoilage of beer by *L. pastorianus* is dependent on an adequate carbohydrate supply, though the addition of sugar may not result in increased acid production if sufficient residual yeast is present to cause rapid fermentation. Increase in hydrogen ion concentration, hop extractives and alcohol all delay the onset of the logarithmic phase of growth. Varieties of *L. pastorianus* are: var. *hindneri*, often known as a distinct species, and var. *berolinensis*. No lactobacilli other than *L. pastorianus* and its varieties have been recorded as beer spoilage organisms, but the evidence suggests that other species will eventually be reported.

Of the coccus types, *Streptococcus acidi-lactici* grows singly or as short chains and sours unhopped wort. This species and the very similar *Str. hindneri* are apparently of scant importance to the brewing industry. Beer spoilage cocci include those forming tetrads and, this feature of morphology has attracted overmuch attention. The resulting confusion has led to the names *Sarcina*, *Pediococcus* and

Tetracoccus being applied to this group. The author's evidence indicates that the tetrads are merely features often exhibited by streptococci, apparently caused by the clumping of two pairs of cells. The beer streptococci are homofermentative and are responsible for the so-called "sarcina sickness." The characteristic odour accompanying this disease is due to the production of diacetyl by the organism. Inactive lactic acid is produced from carbohydrates. The members of the genus *Streptococcus* infecting beer are: *Str. damnosus*, *Str. salicinaceus*, *Str. pentosaceus*, *Str. viscosus* and *Str. limosus*; the last four are to be regarded as varieties of *Str. damnosus*. Whilst *Str. damnosus* produces typical sarcina sickness, the varieties *viscosus* and *limosus* are rope-producing organisms, the former only manifesting this phenomenon in the presence of carbon dioxide and readily losing its rope producing propensities on subculture. The variety *limosus* produces rope from glucose, fructose, maltose and sucrose and not from dextrin which is utilized by *Acetobacter*. Beer streptococci are unaffected by high concentrations of alcohol (e.g., 8 per cent.); they will grow even in very low gravity beers and towards hops behave similarly to *L. pastorianus*.

A. E. W.

III.—BEER

Apparatus for Transmission Turbidity of Slightly Hazy Materials. R. B. BARNES and C. R. STOCK (*Analyt. Chem.*, 1949, **21**, 181-184). The measurement of small degrees of turbidity may best be done by an instrument in which the unscattered light is discarded, leaving substantially all the scattered fraction. In the apparatus here described a sharply defined beam of light passes normally through a layer of the solid or liquid under examination, which is placed against a hole centred on a horizontal axis of a hollow sphere whose interior surface is painted matt white. The unscattered light passes along this axis to a second hole on the opposite side through which the beam can pass, but which can also be closed to retain all the light within the sphere. The brightness of the sphere wall is recorded by a galvanometer connected to a number of photocells inside the sphere. To obtain a measurement the sample is placed in position, the exit hole of the sphere closed and the galvanometer deflection, D_1 , read at a certain shunt factor, S_1 . The exit hole is then opened and a second deflection, D_2 , is read with a higher sensitivity shunt setting, S_2 . The turbidity is defined, in per cent., as $100 D_2 S_1 / D_1 S_2 - T_i$, where T_i is the instrument error previously determined (for the instrument alone in the case of solids, or for instrument plus cell where liquids are examined). The speed with

which the two readings can be taken makes it unnecessary to consider fatigue of the photocells, or any except rapid changes in the material being measured. The mode of operation makes each sample, in essence, its own standard, and eliminates almost entirely the problem of colour compensation. Full details of the construction of the apparatus and its adjustment and operation are given. Among the examples of its use which are cited are observation of the rates of growth of bacterial cultures and comparison of the clarities of beers.

A. A. D. C.

Organoleptic Tests in the Food Industry. III. Statistical Considerations in Organoleptic Tests.

E. C. WOOD (*J. Soc. chem. Ind.*, 1949, **68**, 128-131). If it is desired to know whether a sample B from a batch of food is superior or inferior to the standard A, it is obviously unwise to rely on the opinion of one individual tasting only one sample of each, for there is a 50 : 50 chance of guessing the correct answer. Mathematically, there is a 0.5 probability of obtaining the correct answer by chance. If two samples of A and two of B are offered, unlabelled and with similar presentation, there are now six possible ways of identifying a pair. In this case the correct answer may be obtained by chance only one in six times (probability 0.17). If three samples of each are taken the probability of chance selection of the correct combination is reduced to 0.05 and the statistician usually accepts this figure as significant. If action is based on a determination of this nature, not more than 5 per cent. of the decisions will be incorrect, but if a large sum of money is dependent on the decision the number of samples may be increased further. Similar results are achieved by increasing the number of observers. If x observers decide between two samples, there are $2x$ possible combinations of answers. In such a case observers can agree by chance 1 in $2x$ times and for such a result to be significant x should be five or greater. When many observers are to taste and assign scores to several samples, a different technique, *analysis of variance* must be used. The underlying principles of the latter are to be found in statistical textbooks, but the method is to be applied with caution as the assignment of arbitrary scores may lead to some errors. It is therefore, sometimes more desirable to ask the observers merely to place samples in order of preference. In this case the results cannot be treated by the *analysis of variance* method, but a set of figures is obtained representing the general superiority of one sample to another.

A. E. W.

Practical Approach to Reducing Air in Beer. A. C. SMITH, jun. (*Amer. Brewer*, Feb., 1949, 19-22, 75). In order to discover why

bottled ale contained less air than lager beer, both of which were finished and bottled under similar conditions in the same plant, the author determined the air content of a large number of samples from the fermenting vessel onwards by means of the Schwarz tester. The results obtained show that during the early stages of fermentation the air content of the wort diminishes to a minimum, but in lager after about 6 days the amount gradually increases by absorption from the atmosphere; with a top fermentation the compact yeast head prevents this. If fermented in a closed fermentation vessel lager, even after 11 days, contains only 2.5 c.c. of air *per* litre, about a quarter of that found in the same beer from an open vessel. Carbonation has little effect on the air content if the carbon dioxide is pure, but if air top pressure is adopted at a later stage the amount may rise by about 4 c.c. *per* litre. During bottling a further small quantity is taken up in the bottling tank, the amount increasing with the time the beer remains in this vessel. In the filled bottles knocking eliminates slightly more air than does jetting, presumably because the latter affects only the upper portion of the beer. Attempts made to remove air by over-carbonating to 3,000 c.c. *per* litre and releasing the pressure at intervals down to 2,600 c.c. *per* litre met with little success, and such a procedure is liable to produce excessive foaming. From his results the author concludes that to reach an optimum of about 3 c.c. of air *per* litre of beer, closed fermenting vessels should be used, the carbon dioxide should have a purity of 99.95 *per cent.*, and this gas should be used for top pressure instead of air, whilst filled bottles should be induced to foam by knocking instead of jetting before being closed. T. J. W.

IV.—PLANT, MACHINERY AND BY-PRODUCTS

The Use of Instruments in the Treatment of Brewery Waste. A. O. PEARSON (*Brewers' Digest*, 1949, 36, No. 2, 47-49). The disposal of brewery wastes constitutes a difficult problem owing to their varied nature and the legislative restrictions imposed in many places. In order to simplify the treatment of liquid wastes before running these into the sewers, various modern instruments have been applied in order to render the processes automatic. For the control of p_H suitable electrodes are fixed in tanks or pipe lines and connected to an electronic-pneumatic controller which automatically controls the addition of a reagent for adjustment to the required value. To ensure complete mixing, the liquids are violently agitated before reaching the electrodes. Many forms of flow-meter for determining the total effluent, or that in various portions of the plant, have been devised, but

those most commonly used are the differential head type and Parshall Flumes, the former being operated either by a Pitot tube, a Venturi tube or an orifice plate. Each of these elements may be adapted to indicate, record, integrate or control the flow at different points in the plant. An important factor in the purification of brewery sewage for disposal is the digestion of sludge and this is achieved most satisfactorily by treatment in heated, covered tanks followed by air drying on sand beds. Digestion is carried out between 80° and 90° F., and the rate of flow, temperature, supply of heating water, fluid level, *etc.*, may be regulated and recorded automatically by suitable instruments. The treatment of brewery waste has become of great importance recently and many instruments in addition to those mentioned above which have proved of service in other directions may be applied for this purpose. T. J. W.

Solubility Studies on the Nitrogenous Constituents of Spent Brewers' Grains.

B. VASSEL, S. J. STEPHENS and P. R. CELUSTA-HOWARD (*Cereal Chem.*, 1949, 26, 182-186). The authors have attempted to extract the nitrogenous materials from brewers' grains by grinding in a ball mill and extracting the powder with various solvents, including dilute acids and alkalis and salt solutions for various periods. Of these the most effective were alkaline solutions, but several hours' contact was necessary before most of the proteins were extracted and, in addition, much of the carbohydrate material was rendered soluble. The most favourable results showed that the original 4.13 *per cent.* of nitrogen in the grains was raised only to 4.75 *per cent.* in the finished product. It is suggested that the low solubility is due to the high temperatures used in drying the grains, and probably drying at lower temperatures would result in less insolubilization of the proteins and thus facilitate subsequent extraction. T. J. W.

V.—BIOCHEMISTRY

Activity of α - and β -Amylases. I. A. PREECE and M. SHADAKSHARASWAMY (*Biochem. J.*, 1949, 44, 270-274). Recent proposals for the determination of malt α -amylase (this *Journ.*, 1947, 154; 1948, 141) involve the assumptions that the reactions of α - and β -amylases are additive and that conversion conditions are such that production of reducing groups bears a linear relationship to enzyme concentration and time of action. The problem has been investigated to test these assumptions. Conversions of 1 and 2 *per cent.* soluble starch solutions were carried out at p_H 4.6 and 21° C., the reaction being stopped by addition of sodium hydroxide solution to p_H 10.5-11. Reducing groups were determined by the

Hagedorn-Jensen method (this *Journ.*, 1947, 216) extended up to a maximum of 18 mgrm. of reducing groups. β -amylase was prepared from barley by a modification of the method of Hopkins *et al.* (this *Journ.*, 1940, 507) and contained not more α -amylase than would produce 1/750 of the saccharification due to β -amylase. α -Amylase was prepared from a malt dried at not higher than 45° C. The ground malt was extracted for 3 hr. with 20 *per cent.* (by volume) alcohol and the filtrate adjusted to 60 *per cent.* alcohol. The precipitate was taken to dryness, redissolved in water and treated at 70° C. for 15 min. with 0.2 gm. calcium acetate *per* 100 ml. After cooling and removal of coagulated material, the α -amylase was re-precipitated and taken to dryness. This preparation possessed no β -amylase activity.

There is a linear relationship between time and reducing-group production in 1 and 2 *per cent.* starch conversions with β -amylase up to 15 *per cent.* hydrolysis and with α - and β -amylases, acting together, up to 20–25 *per cent.* hydrolysis. With α -amylase, true linearity is lacking, but there is approximate linearity up to 10–15 *per cent.* hydrolysis, so long as the reaction time does not exceed 30–45 min. The additive relation holds during the linear phase of joint action, so that assumptions of linearity of joint α - and β -amylase action up to 40 *per cent.* hydrolysis made in applications of Kjeldahl's law are not justified. The joint action of the two enzymes is truly additive up to 20–25 *per cent.* hydrolysis for β : α ratios varying from 4:1 to 1:4 (in brewery malt extracts this ratio is usually about 4:1). For the determination of β -, α - and joint amylase activity (diastatic power) by the ferricyanide technique, hydrolyses should be carried out within 30 min. and the theoretical maltose produced should not exceed 15, 10–15 and 20–25 *per cent.* respectively. C. R.

Crystalline Derivatives of Isomaltose.

M. L. WOLFROM, L. W. GEORGES and I. L. MILLER (*J. Amer. chem. Soc.*, 1949, 71, 125–127) Studies on starch structure have necessitated the preparation of a reference compound made up of two D-glucose residues combined by a 6- α -D-glucosyl linkage. The dextran, synthesized by *Leuconostoc dextranicum* from sucrose, has been used as a source of such a disaccharide, since it is bound largely by such linkages. Controlled hydrolysis of the dextran was carried out under conditions not favouring reversion. The dextran was dissolved by shaking with 30 *per cent.* hydrochloric acid and held at 24–26° C. until the specific rotation had fallen to +105° (about 12 hr.). The solution was then poured into 5 volumes of water, the acid neutralized with basic lead carbonate, filtered and the lead removed from the filtrate

by H₂S. After taking to p_H 7.0 with sodium bicarbonate and concentrating *in vacuo*, the remaining ionic material was removed by passage through synthetic resins; the solution was then concentrated to a syrup at 50° C. and this dried to an amorphous powder by azeotropic distillation with absolute alcohol under reduced pressure. This powder was acetylated with acetic anhydride and sodium acetate and the octa-acetate separated chromatographically from a benzene solution of the amorphous acetylated product, using a Magnesol-Celite column and developing with benzene-ethanol (100:1). The zone, one-third of a column length from the bottom, was cut off, eluted with acetone and evaporated to a syrup. The chromatographic separation was repeated on a benzene solution of this syrup. The product was recrystallized from ethyl alcohol and consisted of 6- α -D-glucopyranosyl- β -D-glucopyranose octa-acetate. The 1,6-linkage of this compound was established by periodate oxidation of the methyl pyranoside of the deacetylated compound. De-acetylation of the octa-acetate yielded the free reducing disaccharide in amorphous form. The disaccharide had $[\alpha]_D^{24} + 103.2^\circ$ (water) and was not fermentable by yeast. C. R.

Osmotic Pressure Studies on Maize Amylose.

F. C. CLEVELAND and R. W. KERR (*J. Amer. chem. Soc.*, 1949, 71, 16–20). To obtain a more accurate knowledge of the molecular size of starch components, osmotic pressure measurements were made on a sample of maize amylose acetate in chloroform, in 2,4-pentanedione, and in pyridine. The amylose was prepared from maize starch by Schoch's method (this *Journ.*, 1943, 153) by Pentasol precipitation from an autoclaved sol. The amylose was separated by means of a super-centrifuge, redissolved in hot water and recrystallized by addition of butanol. The product, evaluated by iodine adsorption, was exceptionally pure. Acetylation was carried out by dispersing in hot, aqueous pyridine, distilling off the water and then acetylating, first with acetic anhydride alone, and then with addition of fused sodium acetate. Solutions for osmotic pressure measurement were made by wetting overnight with part of the solvent, then heating for 15 min., cooling and making up to volume. Measurements were made at 30° C. using membranes of uncoated cellophane, pretreated by soaking for 6–24 hr. in a series of solvent mixtures. Molecular weights, expressed as DP_n values, calculated from the measurements were 455 in chloroform, 675 in pentanedione, and 1,370 in pyridine. Four sub-fractions of another butanol precipitated amylose were prepared by the ethylene diamine-ethyl ether technique.

The acetates of these sub-fractions, in chloroform solution, gave values of 250, 390, 525 and 675, whilst the unfractionated amylose gave 435. The great variation in length of the amylose chains comprising any one sample may explain in part the different values obtained when different systems, *e.g.*, osmotic pressure as opposed to viscosity, are used for molecular weight measurement. The higher *DPn* values found in pyridine and pentanedione solution may be due to association of the acetates in these solvents. The limit of molecular size for solubility in chloroform was shown by tapioca amylose acetate (*DPn* = 1050). C. R.

Differentiation of the Products of Hydrolysis of Potato Starch and of a Linear Fraction from Maize Starch. R. B. ALFIN and M. L. CALDWELL (*J. Amer. chem. Soc.*, 1949, **71**, 128-131). The work deals with the action of highly purified, maltase-free pancreatic amylase on unfractionated potato starch, Lindner soluble starch and a linear maize starch fraction. Hydrolyses were carried out as previously reported (this *Journ.*, 1948, 341), samples being withdrawn at intervals for determination of the reducing value by iodometric titration (this *Journ.*, 1936, 359), which gives also a measure of the number of glucosidic linkages ruptured. The unfractionated and soluble starches were hydrolyzed at the same rates with the same concentrations of enzyme and under similar conditions, but differences occurred between the unfractionated starch and the linear fraction. Using relatively low enzyme concentrations, during the early stages the unfractionated starch was hydrolyzed more rapidly; but, after about 30 *per cent.* of the glucosidic linkages of the substrates had been broken, the linear fraction was hydrolyzed more rapidly. These results suggest that there are more points of attack for pancreatic amylase in the branched chain, but that the reducing dextrans thus formed are hydrolyzed less rapidly than those derived from the straight chain. When equal numbers of glucosidic linkages have been broken in the hydrolyses, the relative concentrations of glucose and maltose differ according to the substrate. Appreciable maltose is present from the earliest stages, but in larger concentrations in the linear hydrolyzate. Glucose is liberated from both substrates; it does not appear in the very early stages in either case, but appears earlier, and in higher concentrations at equivalent hydrolysis, in hydrolyzates of potato starch. These differences suggest that the presence of 1,6- α -D-glycosidic linkages in branched chain components interferes with maltose formation and favours that of glucose. Pancreatic amylase causes rapid breakdown of both substrates to reducing dextrans of low average molecular weight and

relatively high reducing power. Random hydrolysis of both branched and linear components is indicated. C. R.

Preparation and Properties of Amyloheptaose. D. FRENCH, M. L. LEVINE and J. H. PAZUR (*J. Amer. chem. Soc.*, 1949, **71**, 356-358). For starch studies it is desirable to have a reference compound intermediate in chain length between amylose and maltose. Such a compound could be obtained by splitting any one bond of the molecule of Schardinger's β -dextrin (cyclohepta-amylose) (this *Journ.*, 1942, 219), which contains seven glucose units linked by maltose bonds. 100 grm. of pure cyclohepta-amylose, prepared as described elsewhere (see next abstract), dissolved in 400 ml. 0.001 N hydrochloric acid, was refluxed at 99° C. for 7 hr. After neutralization with the calculated quantity of lithium carbonate, unhydrolyzed cyclohepta-amylose was allowed to crystallize out in the refrigerator for 24 hr., filtered off and final traces removed by precipitation with *p*-xylene. The filtrate was concentrated to a thin syrup, and precipitated with four volumes of absolute alcohol. The precipitated syrup (amyloheptaose) was taken up in a little water and reprecipitated five times, and finally dried with absolute alcohol containing a little dry butanol, filtered and washed with dry butanol.

Amyloheptaose was a white, glassy substance, not yet crystallized, very soluble in water and insoluble in alcohol. The specific rotations of two samples in water were +175.5° and 176.5°, the theoretical figure being +179.6° (this *Journ.*, 1943, 80). Molecular weight determination by two methods gave values of 1,200 and 1,206, compared with the theoretical value 1,152. Chromatography failed to detect any glucose, maltose or trisaccharide in the preparation, which was degraded by β -amylase to two molecules of maltose plus one of trisaccharide. The analytical constants, derived from a study of the kinetics of the hydrolysis of cyclohepta-amylose, and the chemical analysis of the phenylhydrazone and potassium aldonate, agree with those calculated for a heptasaccharide.

C. R.

Preparation and Solubility Characteristics of Alpha, Beta and Gamma Schardinger Dextrans. D. FRENCH, M. L. LEVINE, J. H. PAZUR and E. NORBERG (*J. Amer. chem. Soc.*, 1949, **71**, 353-356). In searching for superior precipitants for Schardinger dextrans, the solubilities of the pure dextrans in water and aqueous propanol, and of dextrin acetates in selected solvents, have been studied. The *macerans* amylase used for preparing the dextrans was prepared by the method of Tilden and Hudson (this *Journ.*, 1939, 616). The enzyme was

proved free from hydrolytic amylases before use. The α -dextrin was obtained by enzymolysis of a 3-5 *per cent.* starch paste for 2-6 conversion periods in the absence of a dextrin precipitant. After concentration, the dextrin was precipitated first as the trichlorethylene complex, then as the propanol complex and finally purified by crystallization from water. The anhydrous product had $[\alpha]_D + 150.5 \pm 0.5^\circ$ (water). The β -dextrin was prepared by enzymolysis for 2-3 periods in the absence of a precipitate and then for 30-50 periods in the presence of toluene and fresh enzyme. The toluene-dextrin complex was precipitated. After clarification and concentration, the dextrin was allowed to crystallize from water. The $[\alpha]_D$ of the anhydrous material was $+ 162.5 \pm 0.5^\circ$. The preparation of the γ -dextrin resembled that of the β -dextrin, except that enzymolysis was carried out the whole time in the absence of a precipitant. After concentration, the dextrin was precipitated with trichloroethylene. After removal of β -dextrin, subsequent purification consisted in precipitation, first with bromobenzene and then with *n*-propanol. Recrystallization was repeated from propanol until α - and β -dextrins were absent. Propanol was removed by boiling in water, the solution concentrated and set aside to crystallize. The $[\alpha]_D$ of the anhydrous γ -dextrin was $+ 177.4 \pm 0.5^\circ$.

All three dextrans were identifiable by their crystalline form from solution in 60 *per cent.* propanol. The tests may be carried out on a microscope slide, the α -dextrin giving blade-shaped needles or hexagonal plates, the β -dextrin parallelograms, and the γ -dextrans square plates or rectangular rods. The solubilities of the dextrans in water were respectively 14.5, 1.85 and 23.2 *gram. per 100 ml.* Figures are given illustrating the reduction in solubility in water saturated with various precipitants. The solubilities in 60 *per cent.* propanol were respectively 0.4-1.2, 0.54 and 0.27 *gram. per 100 ml.* The specific rotations and the solubilities of the acetylated dextrans in toluene, methanol, ethyl and butyl acetate are also recorded.

C. R.

Crystalline Xylan and Mannan. A. P. YUNDT (*J. Amer. chem. Soc.*, 1949, **71**, 757). Isolation is reported of a crystalline xylan from barley straw and birchwood, and a crystalline mannan from ivory nut and slash pine. For the preparation of the xylan the material was hydrolyzed with 0.2 *per cent.* oxalic acid at

100° C., the insoluble portion autoclaved at 120° C., and then extracted with water, when the xylan was obtained as hexagonal platelets with rounded corners, which on purification yielded spherocrystals. The crystals were doubly refractive and gave typical crystalline X-ray diffraction patterns. The barley xylan showed a degree of polymerization of 39 and the birchwood xylan one of 35, and optical rotational measurements indicated a 1,4', β -linkage. Mannan-rich fractions from ivory nut and slash pine readily form supersaturated solutions of 1 *per cent.* concentration from which, after heating for several days at 60-70° C., crystals were obtained which were at first rod-shaped but gradually became dumb-bell shaped. Repeated crystallization produced no change, although the crystals assayed only 50 *per cent.* mannan.

A. A. D. C.

Inactivation and Removal of Proteolytic Enzymes from Amylolytic Preparations.

B. M. DIRKS and B. S. MILLER (*Cereal Chem.*, 1949, **26**, 98-109). Since the presence of proteolytic enzymes in various amylase preparations is believed to be the cause of some abnormal results when the latter are used, the authors have attempted to remove the proteases from various amylase preparations. The materials on which the tests were made included enzyme preparations obtained from moulds, bacteria and malted wheat and barley, and a large number of commercial and laboratory-produced adsorbents were examined. The diluted enzyme extracts were mixed with definite weights of one of the adsorbents, the p_H was determined and adjusted if necessary and the mixtures were stirred at intervals for 30 minutes. After standing the liquid was filtered and the proteolytic and amylolytic activities of the original and the treated solutions were determined. Of the 30 adsorbents tested, very few showed great difference in selectivity and further work was carried out on these using a series of p_H values and the addition of sodium chloride in different amounts. The results obtained showed the most efficient adsorbents for the proteolytic enzymes to be those composed of silicates or with a high silica content, but in all cases some amylolytic activity was lost. The most favourable results were obtained by the addition of sodium chloride to a concentration 25 *per cent.* and adjustment of the p_H value to 10 with fungal and cereal enzymes but with bacterial preparations little adsorption of the proteases occurred. T. J. W.

PATENTS

Malt Kilns. R. ROBY, LTD and J. P. WESSON (Brit. Pat. No. 608,791-2, 21st Sept., 1948). The malt is discharged from the horizontal floor of the kiln by means of a curved metal plate having its lower edge fitted with a strip of flexible material. The plate is suspended from a horizontal axle, extends across the width of the kiln, and is connected to an overhead travelling device with which it moves backwards and forwards. When out of use the plate is elevated by rotation on its axle, thus leaving a free space beneath for the operation of a malt turner which may be worked by the same electric motor. After reaching the end of the kiln the malt may be discharged on to a spiral conveyor. T. J. W.

Recovery of Alcohol from Fermentation Carbon Dioxide. Akties. Dansk Gaerings-Industri, Denmark (Brit. Pat. No. 610,341, 14th Oct., 1948). The carbon dioxide is passed first through a closed vessel filled with fragments of porcelain, glass, coke or other material over which a stream of dilute alcohol is falling and then passes up a similar vessel through which water is falling. The dilute solution of alcohol thus produced is passed through a cooler and thence to the first absorbing vessel. Fresh water is added continuously to the second vessel and an equal volume of solution containing from 3 to 4 *per cent.* of alcohol is run off. The process reduces the alcohol content of the gas from about 25 to 1-3 grms. *per* cubic metre.

T. J. W.

Improved Form of Attenuator. The Aluminium Plant and Vessel Co., Ltd. and S. W. T. Paine, Wandsworth, London (Brit. Pat. No. 608,972, 23rd Sept., 1948). This device consists of a parallel series of metal tubes fitted into a header at each end, the latter being provided with transverse baffles to ensure satisfactory flow of the cooling liquid. Trunnions are fitted at the centres of the headers so that the whole device may be rotated on a horizontal axis through any desired angle in order to facilitate cleaning. Inlet and outlet ports are fitted close together at one corner or alternatively may be arranged to traverse one or both trunnions, thus eliminating disconnection when the system is rotated. T. J. W.

Manufacture of Casks, etc. E. ROBERTS, London (Brit. Pat. No. 608,838, 21st Sept., 1948). Some or all of the staves in a cask or other similar vessel are constructed of two layers of wood. The inner layer is of quartered oak in which the medullary rays are arranged at right angles to the length of the stave whilst the external layer is composed of fitch oak having the medullary rays parallel to the length of the stave. Thus the inner layer has less porosity and greater resistance to internal pressure and the outer casing is more porous but withstands rough usage better. Whilst the stave is being shaped and fitted the two layers are held together temporarily by nails, pegs or a removable adhesive.

T. J. W.

Froth or "Head"-producing Device for Use with Beer Engines. G. E. MORRIS, Worksop, Notts. (Brit. Pat. No. 604,971, 13th July, 1948). This appliance consists of a solid, truncated-conical plug of wood or other suitable material provided with a series of longitudinal or spiral grooves in its surface. A knob is provided at the wide end of the cone to facilitate its insertion into or removal from the outlet nozzle or delivery pipe of the beer engine. Owing to the partial obstruction of the outlet by the cone, a froth or head is produced on the emerging beverage.

T. J. W.

Beer Valves. R. WALSH, Blackburn, Lancs. (Brit. Pat. No. 608,263, 13th Sept., 1948). To eliminate the trouble caused by a particle of solid matter becoming lodged on the seating of the valve in a beer engine pump it is proposed to use one of similar type which is easily opened and cleared. The valve is fitted in the pipeline close to the cask and renders a valve in the pump unnecessary. The device consists of a metal casing containing a poppet or similar valve provided with a rod held in position by means of a hole in a bridge piece. A wing on the upper surface of the valve enables it to be readily lifted out for clearing when necessary. The top of the casing is closed by a cover held in position by a coupling ring which may be removed without disconnecting the pipes.

T. J. W.

REVIEWS

Trace Elements in Food. By G. W. MONIER-WILLIAMS, O.B.E., M.C., M.A., Ph.D., F.R.I.C. Pp. viii + 511. London: Chapman and Hall, Ltd. 1949. Price 30s.

The name of the author will indicate the excellence of this book to a wide circle of analysts in this and other countries. The biologist will perhaps be less familiar with his name and work, although the present volume may arouse his interest at the outset, since the biologist has made the term "trace elements" peculiarly his own. The author, however, is at pains to stress that he uses the term in the sense of those small amounts of elements in one form or another which may occur in food by virtue of their origin in the plant or animal, or in the course of processing. The trace elements of the biologist are often to be regarded as essential or beneficial; the trace elements in the sense employed by Dr. Monier-Williams are unfortunately often toxic, and it is the business of the food chemist to be fully cognizant of their significance.

The present volume deals exhaustively with more than 28 elements and constitutes an encyclopaedia of their occurrence whether in natural sources or as incidentals of manufacture; of their biochemistry and toxicology; and of the many enactments and regulations concerning their presence in food. In addition, their determination is described, and in this section the use of organic reagents is noteworthy. Although the word encyclopaedia has been used, it must not be inferred that the book is in the all too familiar style of a catalogue. It is eminently readable and indeed contains many fascinating and surprising pieces of information, which are fully documented in the matter of references. Literature references are given at the end of each chapter, and throughout the book reach an impressive total.

There is little doubt that this volume will prove a welcome addition to the bookshelves of all chemists in food laboratories, and there is much that chemists in the brewing and allied industries will find valuable. The vigilance of those responsible for the purity of the national beverage is such that the public have nothing to fear from contamination, and the bogies of the turn of the century have long been laid. With the advent of stainless metals and plastics, the possibility of metallic contamination is reduced to a minimum and is scarcely likely to be of a toxic nature. Indeed, it is more likely to be rather of some aesthetic significance, as those who have had to deal with occasional metallic haze will be aware. The occurrence

and determination of undesirable elements in beer, wines and other beverages, in yeast, glucose syrup and other sugar products is discussed in appropriate sections. For those wishing to perform the analysis described, reference to the original papers and other sources will be necessary; but throughout the book the methods are given in fully intelligible outline, together with difficulties, defects and most recent modifications and improvements.

Although the book is produced "in complete uniformity" with the authorized economy standards"—forbidding phrase—the type is clear, large and bold, and the paper of good quality. The reviewer does not claim to have read all of the 511 pages, but has not yet found a misprint. The success of this book would seem to be as assured as the reputation of its author.

F. W. NORRIS.

Water Treatment. By G. V. JAMES, M.Sc., M.B.E., Ph.D., F.R.I.C. Second Edition. Pp. xi + 247, with 27 illustrations. London: The Technical Press, Ltd. 1949. Price 30s.

The first edition of this comprehensive volume was published in 1940 and was reviewed in this *Journal* (1940, 165). The present issue follows the same lines as its predecessor, but the inclusion of new matter has extended the text by 29 pages and the addition of a name index has greatly facilitated reference and increased the value of the book. An introduction dealing in a general manner with the purification of potable waters is followed by chapters devoted to sterilization, the removal of colour, odour and flavour, softening, and the purification from war-time poison gases of supplies for domestic use, on sea-going vessels and for the Army. After descriptions of methods for treatment of swimming bath and boiler waters several chapters are devoted to the waters required in various industries and the treatment and disposal of their waste effluents. The remainder of the volume deals with the important subject of domestic sewage and its purification. The text is in the form of abstracts of scientific papers, patents, official methods, etc., to which in some cases are added the theoretical principles involved, the effects of impurities and other details relevant to the subject. Throughout the book numerical references are given to bibliographies provided at the end of each chapter, thus enabling the reader to ascertain full details from the original sources if required.

The nature and variety of the subject-matter

indicate the great attention that has been directed in many countries to water in various degrees of purity, and form a comprehensive and concise survey of the principal literature up to the present time; thus the book will prove of value to all concerned in the numerous uses of water. The different topics have received rather unequal treatment and undue space has been given to some of little importance. For instance, more than a page has been devoted to the "Luminator" process for the prevention of boiler scale, but in practice this has been proved to be of little or no value.

Much care has been exercised in the compilation of this work for few errors are apparent throughout the text and these are only of minor importance, but the subject-index is less complete than it should be and a few items have been inserted under the wrong letter and are thus liable to be overlooked as indicated in the review of the first edition. The volume is well produced on good paper and the type is easily legible, whilst the price is by no means excessive for a work of this nature which has entailed much labour and patience in its preparation.

T. J. WARD.

Rothamsted Experimental Station, Harpenden:
Report for 1947. 131 pp. St. Albans:
Gibbs & Bamforth, Ltd., 1948. Price 3s.

Although the name of Rothamsted is so widely known, the size of the undertaking and the breadth of work which is there carried out may not be so generally recognized, so that the reading of such a Report as this is very useful to supply a true perspective; the size of the Staff and its distribution among the various Departments is of itself a clear indication of the magnitude of the Station's responsibilities. The reports of individual Departments are preceded by an Introduction by the Director, Dr. W. G. Ogg, in which is given a general survey of what has been accomplished during the period covered. Fifteen Departmental Reports follow, covering physics, chemistry, microbiology, pedology, botany, crop physiology, statistics, plant pathology, biochemistry, entomology, bees, insecticides and fungicides, field experiments, the farms, and Woburn Experimental Station. This list is quoted in full, since otherwise it would be easy to fail to realize how many branches of study are involved in a modern research station; it is important to notice, nevertheless, that there are many examples in the Report of inter-departmental co-operation, so that best advantage is taken of the available facilities. The Chemistry Department's investigations on fertilizer placement are of particular interest; other fertilizer problems and various aspects of soil analysis and character have also been dealt with. The influence of micro-organisms on soil structure has been studied by

the Department of Soil Microbiology, where work has also been done on soil bacteria, actinomycetes and protozoa. Soil problems have also interested the Departments of Pedology and Botany, and it is of interest to know that the Imperial Bureau of Soil Science has been located at Rothamsted since 1929. Barley is one of the crops considered in the Department of Crop Physiology in its analysis of growth and yield of field crops, whilst viruses are receiving attention in the Departments of Plant Pathology and Biochemistry from the points of view of conditions influencing susceptibility and of the binding of virus in the tissues. Long- and short-term experiments continue on the Rothamsted farm and at Woburn. In addition to the Departmental Reports, special reviews are also given on the Electric Charge on Soil Particles and on Potato Virus Diseases. It has only been possible here to mention a few of the topics discussed; it can confidently be recommended to those interested in agricultural and related matters, however remotely, that they should dip into this publication, for they are bound to find much of interest.

The Report concludes with a list of publications made or in preparation during the period under review. This impressive list of 158 items is made the more useful in that the majority of references included are followed by short abstracts of the papers concerned. Altogether this is an excellent and well-produced Report, which fulfils outside its direct object the stimulating purpose of making the reader wish to go on reading.

I. A. PREECE.

Journal of the Royal Agricultural Society of England. Vol. 108. 1947. Pp. 210.
London: John Murray.

This valuable report which is published annually, comprises a series of articles by various authors summarizing the developments and results obtained in agricultural research carried out during the year in the British Empire and elsewhere. These include monographs on plant breeding, soils and fertilizers, farm economics, implements and machinery, feeding and diseases of livestock and dairy farming. Nearly every article is followed by a useful bibliography giving a list of publications to which the reader may refer for more detailed information in the subjects dealt with.

The remainder of the volume is devoted to ten special articles on a wide range of subjects, including *inter alia* land reclamation, timber research, the development and manufacture of farm machinery and agricultural legislation. These contributions not only provide much useful information but the literary styles adopted by the authors add interest and pleasure to their perusal.

The extensive range of subjects dealt with precludes any detailed reference to all in a brief review, but attention may be drawn to a few indirectly related to the brewing industry. To the farmer the details of a new abnormality in barley will be unpleasant news, since it leads to loss by sterility, either partial or total, of the ears. The condition is described as "yawning" and examination of the florets showed that sex reversal had occurred for small ovaries were developed on the filaments instead of anthers bearing pollen. Although the cause of this defect is not definitely known at present, it is certainly not due to the attack of insects or fungi, but appears to bear some relation to the extremes of weather conditions during development of the flowers.

A paragraph is devoted to the new barley placed upon the market last year and known as "Earl." This is a development from the well-known Spratt-Archer and although its conditions for growth and malting and brewing behaviour are similar to those of its parent it ripens from 7 to 10 days earlier.

The section dealing with farm machinery will prove of considerable assistance to those who are endeavouring to select, from the somewhat bewildering variety of implements available, the ones best suited to their requirements.

Now that the use of the combine harvester is increasing the problem of adequate storage is becoming urgent for the farmer as maltsters will probably be unable to accommodate more than half of their requirements. Since both maltsters and millers prefer to do their own drying it is inadvisable for the farmer to instal a hot-air drier, but by the use of a series of small silos with a pipe fitted in the base through which a current of cold or slightly warm air may be pumped for an hour daily, the moisture even of damp barley may be gradually reduced and thus ensure safe storage for several months. It is probable that in future the straw will be in demand so that the cost of combining will tend to approach that of the older method of harvesting more closely than at present.

Reference to the numerous subjects in the text will be greatly facilitated by the index provided at the end of the volume, and the abundance of knowledge surveyed in the report will aid in the liberation of the human race from the food shortage evident in many areas of the world at the present time.

T. J. WARD

Green Crop Drying in Holland, Sweden and Denmark. Pp. 22, with 23 illustrations. London: 1948, His Majesty's Stationery Office. Price 1s. 3d.

In 1947 the shortage of animal feeding stuffs in this country drew attention to the possibility of drying grass for such use, and a Mission of six members visited Holland, Sweden and Denmark to investigate the industry, which is of considerable importance there. The results of this visit are published in Report No. 7 of the Agricultural Overseas series, under government auspices. This brochure includes the history of green crop drying, details of the field equipment and drying plants used, the marketing and use of the product, the organization of the industry and other relevant matters. The extent of the industry may be gauged from the fact that in 1946 Holland produced 25,000 tons of dried products in 139 installations. Although the principal crop dried is grass, successful results have been attained when the same apparatus was used for kale, lucerne, carrots and other vegetable products. Several forms of drying plant are described and the operation of three types is depicted in a very lucid manner by coloured diagrams. One of the forms described is already in use in this country and the members of the Mission recommend that two supplied by other firms should be obtained and tried out as soon as possible under British conditions. Some useful details concerning the operation of three types of drier are given in the form of a table from which we gather that from 3 to 5 operatives are required *per* shift and the man-hours needed *per* ton of dry product vary from 4.85 to 8.99 when calculated to performance in this country, assuming that an original moisture content of about 72 *per cent.* is reduced to 10 *per cent.* Although the temperature of the air current used for drying is over 900° F. no excessive scorching of the product occurs and the proportion of carotene retained in relation to the other constituents is satisfactory. Such an industry as that described, if operated in this country, would go far towards solving the problem of providing food for cattle and horses, especially during the winter, and would conserve much of the grass which at present is not available for food when needed.

T. J. WARD

JOURNAL OF THE INSTITUTE OF BREWING

MONTHLY REVIEW

FIRST INTERNATIONAL CONGRESS OF BIOCHEMISTRY, CAMBRIDGE, 1949

EVEN in a year of Congresses, that organized by the Biochemical Society under the Presidency of Prof. A. C. Chibnall must remain memorable. Held at Cambridge from 19th—25th August, a membership of 1,741 was attracted, representing no less than 42 countries; truly, therefore, an International gathering. The programme of the Congress allowed for the reading of some 450 short papers covering all aspects of biochemistry, and for this purpose the proceedings were divided into 12 Sections; with the Sections meeting simultaneously, selection of what it was desired to hear was essential, and a bulky volume of abstracts in the possession of each member assisted in making the choice.

However, for the fermentation biochemist, choice was frequently difficult; thus, he might find himself interested at one and the same time in the proceedings of Section II (Microbiological Chemistry) Section IV (Proteins), Section XI (Plant Biochemistry), or Section XII (Industrial Fermentations), or even those of other sections with more remote-sounding titles, but some of whose contributions were nevertheless closely related to his own special interests. The difficulty of inevitable overlap was met in a number of cases by the holding of joint sessions between certain of the Sections. Industrial Fermentations, under the able chairmanship of Mr. H. J. Bunker, attracted good attendances and covered a wide field, including brewing and yeast production, the production of antibiotics, panary fermentation, ensilage, and fermentations resulting in the production of such materials as 2,3-butanediol, butyl alcohol and acetone. Of particular interest to brewers would be T. K. Walker's review of lactic acid bacteria as infections in brewery

products, providing as it did a systematic discussion of the characters and potential dangers of organisms of this group. E. C. Barton-Wright dealt with the nitrogen metabolism of yeasts, showing certain differences in behaviour towards amino-acids by top and bottom yeasts; polypeptide nitrogen was stated to be of special importance to top yeasts. This paper aroused a particularly good discussion.

A number of other papers also dealt with yeast. C. Rainbow reported on strains of brewers' yeast which fail to grow in the absence of *p*-aminobenzoic acid, this substance being probably involved in the synthesis of methionine and histidine. E. R. Dawson and J. G. Harrison discussed the importance of biotin in the production of bakers' yeast, whilst D. J. Munns and J. White dealt with yeast nutrilites more generally. A practical paper of importance was contributed by B. Trolle, who has studied the relation between the amount of yeast in suspension in wort and the carbon dioxide evolution during fermentation; it appears to be confirmed that a relationship exists, though its exact terms are of some complexity.

Two papers which were read successively and discussed together were descriptions of work carried out by R. H. Hopkins and J. H. St. Johnston, respectively, on wort proteins. It is clear that considerable difficulty exists in the separation of well-defined protein fractions from wort, but work of the type described by these authors should serve to focus extra attention on the problem, and when further information is available it is probable that differences in opinion which exist in relation to the interpretation of results will be capable of resolution. Proteins in brewing was also the subject of E. Sandegren, who has been associated with extremely important work leading to the isolation of an albumin and four globulins from barley. The

albumin carries the amylase activity, and β -globulin appears to be the fraction which yields chill haze (and probably also oxidation haze) materials to beer. Means were discussed for controlling the amount of these proteins in wort, and the interest taken in the paper was evidenced by the discussion which followed.

Starch and amylases were discussed in Sections VI and XI jointly, under the chairmanship of C. F. Cori, and it was a great pleasure to hear papers on these topics by such well-known authorities as K. H. Meyer, S. Peat and E. J. Bourne, K. Myrbäck, and E. H. Fischer. The discussion which followed was of such interest that, thanks to C. S. Hanes, a separate informal session was arranged for a subsequent afternoon so that it might be continued, and the questions of amylolytic degradation and of starch synthesis were very profitably dealt with by the exponents mentioned—a memorable occasion. It should be mentioned that a paper on starch was also given in Section XII by J. Blom and B. Schwarz, who showed how it is possible to use potato starch in the determination of diastatic activity, a treatment in a Turmix or Waring-Blendor being necessary to prepare the starch for use. It is claimed that greater precision is possible by this means than when soluble starch—a very variable commodity—is used.

A joint session of Sections VI and XI also discussed the plant cell wall, with A. G. Norman presiding. Of the various papers presented, that of F. A. Isherwood on the chemical constitution of cell-wall polysaccharides presented features of special interest; a study of polysaccharides from the pear has led to the conclusion that only a limited number of polysaccharides exist, and each on hydrolysis yields (from this source at least) a single sugar. Similarly, I. A. Preece and A. S. Ashworth (in Section XII) had reported a study of barley polysaccharides, and while their products on hydrolysis gave mixtures of sugars, they also expressed the belief that only a limited number of types of polysaccharide molecule are involved, though they considered that each might exist at numerous levels of molecular complexity. This is an important problem in relation to malting changes. Other topics from Section XI which may be mentioned include A. C. Hulme's study of apple protein, which has led to the view that the bulk of this protein

represents enzymes, including oxidase, invertase, protease, and amylase; the means developed for the isolation of protein fractions from this source may well prove useful in other connections. F. Wokes, C. Klatzkin and F. W. Norris, discussing the biochemistry of germination, dealt with changes in content of vitamins of group B during the germination of seeds, especially in relation to malting loss and the utilization of carbohydrates and proteins as sources of energy for the germination process.

These then represent a few of the papers of actual or potential brewing interest, and the reviewer—like the Congress member—must be content to make a selection of topics. However, there were additionally numerous functions where the restriction of competition did not operate. Amongst the most impressive of these was the Congregation in Senate House, when the Degree of Doctor of Science (*honoris causa*) was conferred upon Viscount Addison (Lord Privy Seal), Prof. C. F. Cori, Sir Charles Harington, Dr. K. Linderstrøm-Land, Prof. A. W. K. Tiselius and Dr. J. Trefouël. Three at least of these names will be immediately familiar to anyone who has made a study of biochemical problems in relation to fermentation. Cori's work at Washington on sugar phosphates and on enzymes has been of outstanding significance both for plant and animal biochemistry, Linderstrøm-Land is Director of the famous Carlsberg Laboratories at Copenhagen, whilst Tiselius has given new techniques to investigators in these fields and the University of Uppsala is in the forefront of physico-chemical advances. Trefouël is Director of the Pasteur Institute and Harington of the National Institute for Medical Research; Viscount Addison is Chairman of the Medical Research Council. Thus, the honorary graduands are not only internationally representative, but also cover all branches of Biochemistry. Academic events of considerable importance were the three special Congress Lectures, given before very large audiences in the Congress Hall. Prof. Cori spoke on the "Influence of Hormones on Enzymatic Reactions," Prof. M. Florkin (Belgium) on "Biochemical Aspects of some Biological Concepts," and Sir Robert Robinson (Great Britain) on "Tryptophan and its Structural Relatives."

Wide and varied social activities were also planned both for members of the Congress

and for their guests; they included, in addition to visits to beauty spots, works, and laboratories, a Ladies' Garden Party in Trinity College, a Garden Party given by Prof. and Mrs. Chibnall in St. John's College, and a memorable reception at Trinity College by invitation of H. M. Government where the company was received by The Lord Privy Seal and Viscountess Addison. A name frequently heard during the Congress was that of Sir Frederick Gowland Hopkins, a name inseparable from Biochemistry and Cambridge. It was a happy idea, therefore, to present each Congress member with a copy of an admirably produced commemorative volume *Hopkins and Biochemistry* (Cambridge: W. Heffer & Sons, Ltd., 1949), edited by J. Needham and E. Baldwin, and described as containing "Papers concerning Sir Frederick Gowland Hopkins, O.M., P.R.S., with a Selection of his Addresses and a Bibliography of his Publications." This is a most fascinating compilation, and forms a worthy tribute to a great man.

The organization of the Congress was excellent. It has been said before but is no less true, that a principal function of such gatherings is to allow people to meet—not only in lecture rooms but also informally as in the Cambridge Sectional Dinners, Garden Parties and Receptions, in Congress Hall and buffet, or even if they so desired in their own rooms. Such contacts are invaluable, and create a store of understanding and good-will which must inevitably be of the greatest possible value. There is another advantage: those working in a restricted field of Biochemistry must have after a Congress of this magnitude a renewed realization of the immense field which the subject covers. They will thus be helped to avoid narrowness of outlook, they may even learn that workers in other branches can often have something to offer them in the way of new techniques. Sectionalization for lectures is essential in a project of this size; it was encouraging, however, to note the degree of selection which so many members exercised in passing from one Section to another, rather than remaining throughout at one pre-selected point.

* * * *

WEST MIDLANDS' HOPS

SUCH is the importance usually attached to

Kent and the South-Eastern Counties as sources of English hops, that the magnitude of the contribution made by the West Midlands is apt to be overlooked. Nevertheless, this area provided nearly one-third of the total hop-growing acreage in 1948; thus, out of a total of 22,787 acres, Herefordshire provided 4,737 and Worcestershire 2,200, a total for the West Midland area of 6,937 acres. These figures are given by E. H. Wilkinson in an article contributed to an interesting West Midlands number of *Agriculture* (July, 1949, pp. 160-162), where it is also stated that the principle varieties grown are Fuggles, Bramling, Early Bird and Mathon, though encouraging results have also been obtained with such new varieties as Brewer's Gold, Bullion, and Northern Brewer. Reasons for the concentration of hop-growing in relatively limited areas are often discussed; the importance of this area is said to be due to the deep rich soil, specialized local knowledge, and the proximity of the industrial regions of South Wales and the Black Country from whence come the annual migrations of pickers. Hand picking still predominates, but some growers are using machines exclusively and although this may solve some problems, there are those who will wonder whether others will not thereby be created.

There appears to be some segregation of varieties in the area: Fuggles represent some 75 *per cent.* of the total acreage and flourish particularly in the heavy soils of Herefordshire; Goldings, on the other hand, are particularly favoured by the deep alluvial deposits of the Teme valley. Problems of hop diseases are important here, as elsewhere, but promising results have been obtained in attempts to propagate true-to-type, disease-free plants by layering and from soft-wood cuttings. *Verticillium* wilt is found in its fluctuating form in a few hop yards, but the progressive form is apparently absent; virus diseases cause the greatest amount of loss. Nettlehead attacks all commercial varieties; but Fuggles, most Wye seedling varieties, and some males are symptomless carriers of mosaic. Whilst the latter disease is apparently not advancing, nettlehead is becoming more widespread and is giving rise to increased anxiety. As a short, summarized account of hop-growing problems, this article is well worth reading.

GRAIN RESEARCH IN CANADA

A PUBLICATION of great interest which has just been received is the *Twenty-Second Annual Report* (1948) of the Board of Grain Commissioners, Grain Research Laboratory, Winnipeg (Ottawa: Edmond Cloutier, 1949, 104 pp.). Members of an industry interested mainly in barley may think that this cereal occupies relatively few pages in the *Report*; nevertheless, it is worth emphasizing strongly that while malting barley may present its own specialized problems, no investigation on other cereals should be passed over lightly, as a consideration of the results obtained may provide a useful clue to some aspect of barley chemistry or behaviour which has hitherto escaped direct solution. However, it is not proposed to discuss the findings of this *Report* on wheat, oats, or the like; one or two comments on barley itself are of sufficient interest to pass on in some detail. Thus, on the agricultural side, there is the warning that excessive use of selective weed killers may impair the germination, particularly of small barley grains. Again, in relation to the "malting quality" of barley, the following quotation may be made.

"For practical purposes, malting quality in barley should be defined wholly in terms of properties that can be measured objectively. . . . But malting quality cannot thus be adequately defined, for, by analysis alone, ways of determining how barley will behave when malted have not yet been devised."

The Canadian practice is to malt and mash all test samples under standard conditions; a reasonable estimate of malting value is obtained from analyses of barley, malt, and wort, from the malting loss, and from observations of the growth of the barley sample. At the same time, the doubt is expressed whether it would not be better to malt each barley sample under conditions varying according to its own character, rather than use merely standardized methods. Naturally, however, practicability and the time factor must be taken into account. Breeding experiments are in progress to produce new varieties giving higher yield, enhanced resistance to disease, other desirable agronomic qualities, and malting qualities "defined in terms of a standard variety which maltsters favour."

The opinion has steadily been developing

that viscosity gives a useful assessment of wort character, but the nature of the material responsible for the viscosity has not so far been determined with certainty; both proteins and polysaccharides (barley gums) have previously been suggested as responsible agents. The indications now are that the main factor is a polysaccharide, and further investigations on the material are awaited with interest. These are matters of general interest, of importance to brewers throughout the world. Another section of the *Report* deals with domestic crop data for the 1947-48 crop year; probably the greatest interest for readers in this country will be the types of data used for analytical assessment, and the means of comparing the samples from the different provinces.

* * * *

THE SECTIONS

LONDON SECTION

THE programme for the Season 1949-50 is announced as follows:

1949.

10th October: *Sterile Bottling*, by Mr. C. Osgood.

24th October: *Microbial Synthesis of Fats*, by Dr. H. Lundin.

14th November: *English Barleys*, by Mr. H. Leslie Thompson.

12th December: *European Brewery Convention Congress*, 1949, by Mr. B. M. Brown.

1950.

9th January: *Comments on the 1949 Hop Crop*, by Lt.-Col. F. N. Richardson.

13th February: *The Research Foundation and its Place in the Industry*, by Sir Ian Heilbron, D.S.O., F.R.S.

13th March: *Liquor Treatment*, by Mr. J. W. Green.

17th April: *Observations on Glass Washing in Public Houses*, by Mr. H. J. Bunker.

The first and last of these meetings are to be held jointly with the Incorporated Brewers' Guild; that of 24th October is to be a joint meeting with the Food Group, Society of Chemical Industry. All meetings are held at the Horse Shoe Hotel, Tottenham

Court Road, London, W.1, at 6 p.m. The Hon. Secretary of the Section wishes it to be known that a cordial invitation to attend these meetings is extended to members of other Sections who may be in London at times when meetings are being held.

MIDLAND COUNTIES SECTION

THE full programme of the Section for the Season 1949-50 is announced as follows:

1949.

15th September: Visit to hop farms in Herefordshire.

22nd September: *The Season's Barley*, by Mr. E. P. Wright.

20th October: *Cask Timbers*, by Mr. E. Doyle and Mr. W. J. Bull (Midland Hotel, Birmingham; 6.30 p.m.).

24th November: *The Season's Hops*, by Lt.-Col. F. N. Richardson.

16th December: Annual Dinner (Midland Hotel, Birmingham; 7 p.m.).

1950.

19th January: *A New Malting Technique*, by Mr. W. Hynard.

23rd February: *Hops—from Grower to Brewer*, by Capt. C. Edwards.

23rd March: *Brewery Water Supply*, by Prof. F. Shotton.

Unless otherwise stated, the meetings are held at the White Horse Hotel, Congreve Street, Birmingham, at 6.30 p.m.

At the meeting of 22nd September, under the Chairmanship of Mr. B. Wood, Mr. E. P. Wright in his paper *The Season's Barley* said that there had been a great variation in yield and quality. The corn had the benefit of a very good autumn and spring seed bed, but the later prolonged dry weather caused many crops to die prematurely, giving in these cases poor quality, low yielding, and highly-nitrogenous barleys. The autumn-sown and early spring-sown barley stood up to the dry weather well because the good early growth covered the land and prevented excessive evaporation of moisture, and the good root development enabled sufficient moisture to be passed to the growing corn, resulting in high yields of reasonable quality, sufficient for the requirements of the brewing trade, and with a nitrogen content of 1.3-1.5 *per cent.* The

very dry weather at harvest and the increased number of combine harvesters enabled the crop to be gathered in record time, and there is no fear of barley still in farmers' barns deteriorating because of high moisture content, as most of the grain had only 12-14 *per cent.* of moisture. The Ministry of Food had been a very large buyer of the barley which could not be absorbed by the brewing and malting trades, and less barley than usual had been stacked, but no doubt it would be ample for the balance of consumers' requirements. There had been more of the Danish types of barley grown because of their high yielding and early maturing qualities, but generally these barleys were not of good malting quality and were absorbed by the Ministry.

* * * *

THE 1949 HOP CROP

BY SYDNEY MYER

A FAIRLY good idea can now be formed of this season's yield. Drought conditions prevented full development of the cones, but while this no doubt reduced the total weight, it has by no means been a drawback for quality. The strig is rather finer and more delicate than normally; the hops are more inclined to be brittle, accentuated by the fact that drying generally is likely to be somewhat on the high side, a result usual in seasons of this type, particularly following a year when the number of "all faults" was extremely high. It rather seems that of recent years oast management has not received as much care as it did, for despite the greater temperature control now available the number of mistakes in curing has been on the increase. Also, in the next stage—probably the most important—when the hops come off the kiln and lie on the cooling floor, sufficient attention does not seem to be given as to when they should be bagged, determined as this should be by atmospheric and other conditions prevailing. Fortunately there has been little late blight, and efforts to control spider have been fairly successful in a season unusually favourable for its development.

Difficulty in estimating yield is increased by great variations from one garden to another, even in close vicinity. The drought, the dominating factor, has of course affected some gardens to a greater extent than others.

The Weald of Kent, where there is normally less depth of soil and where the crop is nearly all Fuggles (which do best in a wet season), will certainly show a considerable reduction on last year, perhaps to the extent of 20 *per cent.* Mid-Kent may not be quite as heavily hit, and East Kent, with its high percentage of Goldings may produce a little more than in 1948, but it must be remembered that the yield in that year was very low. Sussex may produce 85 *per cent.* of last season's figure, while Farnham reports suggest a somewhat similar reduction. In the Western District, Hereford is likely to show an even greater reduction, but Worcester may record a little better result, due to some good crops in the Teme valley.

Trying to make a close estimate, consideration must be given to the grubbing up of 591 acres; against this 721 acres have come into full bearing (third year since planting) and 803 part bearing (second year). It would appear that the reduced acreage thus cancels out, implying a considerable cut (over 10 *per cent.*) of the figure given as brewers' requirements, which with the reduction in beer consumption and stocks held, can be accepted without many regrets. A figure of 220,000 *cwt.*, or not quite 10 *cwt.* *per acre*—the lowest return *per acre* since 1932, may not be far out. The quantity mentioned does not include hops grown by brewers. As hops weigh well this season, the accepted average weight of 1½ *cwt.* *per pocket* may be misleading if the total crop is calculated on this basis. The average net weight is likely to be nearer 1 *cwt.* 2 *qr.* 5 *lb.* The 1949 crop which, as a whole is a much better one than its predecessor, cannot be described as a vintage one. There will, however, be a good proportion of really fine quality with, it is confidently expected, a much higher preservative value than in 1948. 28th September, 1949.

CORRESPONDENCE

COLLEGE OF TECHNOLOGY,

MANCHESTER.

20th September, 1949.

THE EDITOR,

THE JOURNAL OF THE INSTITUTE OF
BREWING.

DEGRADATION PRODUCTS OF LUPULONE

DEAR SIR,—Many years ago and before the publication of the work of Wieland and his

colleagues on the constitutions of humulone and lupulone, the writer commenced a study of the chemical degradation of the latter substance. In the course of this work two isomeric fission products of lupulone were isolated. These were considered to have the empirical formula $C_{18}H_{26}O_4$ (this *Journ.*, 1924, 712). Later, it was discovered that these isomeric substances contained a larger percentage of carbon than was represented by $C_{18}H_{26}O_4$. It was established that the correct empirical formula was $C_{21}H_{30}O_4$.

(Found, for compound of M.P. 91°: C, 72.5, 72.65, 72.7 *per cent.*

H, 8.8, 8.6, 8.7 *per cent.*

Molecular weight (Rast), 351, 322.

Found, for compound of M.P. 169°: C, 72.7, 72.4, 72.4, 72.7, 72.55 *per cent.*

H, 8.5, 8.7, 8.6, 8.6, 8.5 *per cent.*

Molecular weight (Rast), 344, 355, 313, 323.

$C_{21}H_{30}O_4$ requires: C, 72.80 *per cent.*

H, 8.67 *per cent.*

Molecular weight, 346.)

The low values found for carbon in the earlier analyses were traced to low gas pressure (due to a national shortage of fuel at the time the combustions were performed.). This had caused a constant error in the estimations.

The two substances, each of empirical composition $C_{21}H_{30}O_4$, have been subjected to various chemical treatments designed to throw light on their structures and have yielded crystalline derivatives and degradation products. It was intended to undertake further experiments before publication of the results, but pressure of more urgent work delayed completion of the enquiry and the writer has now learned that Prof. F. Govaert and Dr. M. Verzele of Ghent University have carried out recently a study of the constitution of lupulone. In the circumstances it has been thought desirable to publish this brief note and it is hoped that it will soon be possible to communicate the details of the experiments. The writer's thanks are due to Prof. Govaert and to Dr. Verzele for informing him of their investigations.

Yours faithfully,

T. K. WALKER.

THE INSTITUTE OF BREWING EXAMINATIONS: JUNE, 1949

FIRST ASSOCIATESHIP EXAMINATION

CHEMISTRY (INORGANIC AND PHYSICAL)

(Six questions only to be attempted. Two at least of those marked with an asterisk must be included. Formulæ and equations should be used when possible.)

- *1. Give in brief outline *three* of the general methods by which metals are extracted from their ores. What principles decide which of these methods are suitable for particular metals?
- *2. What is the meaning of the expression " p_H of a solution"? With the help of diagrams, show how p_H varies during the course of a titration of (a) a strong acid with a strong base; (b) a weak acid with a strong base; (c) a weak base with a strong acid. What indicators would be suitable in order to identify the end-points in these titrations?
- *3. Give a short account of the periodic scheme for the classification of the elements. Select a group of elements and show how this arrangement fits in with their physical and chemical properties.
4. What is the Solubility Product Principle? Show that it is based upon the Law of Mass Action and give examples of its application in (a) the preparation of a salt in a high state of purity; (b) the precipitation of metallic sulphides in qualitative analysis; (c) the volumetric estimation of chlorides.
5. Describe briefly the manufacture, uses and properties of *three* of the following:—sodium bicarbonate, hydrogen peroxide, bleaching powder, calcium carbide.
6. Give a concise statement of Le Chatelier's Principle of Mobile Equilibrium. Select *two* examples of industrially important reactions and show how the conditions of temperature and pressure under which they are carried out illustrate the operation of this principle.
7. Write an essay on industrial fuel gases, with special reference to producer gas and water gas. Why can water gas not be made by a continuously operating process?
8. Describe and explain *one* method by which the molecular weight of a substance in solution may be determined. Certain substances show anomalous results, the apparent molecular weight being either higher or lower than the formula weight. How may these anomalies be explained?
9. What is the meaning of the expression "hardness of water"? How does hardness of water arise, what are its effects and how may it be estimated analytically? Give a brief account of *two* methods of water

softening which are used on the industrial scale.

CHEMISTRY (ORGANIC)

(Five questions only to be attempted.)

1. Write out the structural formulæ of the monobasic carboxylic acids having the molecular formula $C_3H_6O_3$. Compare and contrast the properties of the compounds represented.
2. If you were given a specimen of a pure, crystalline organic compound, what steps could you take towards identifying it in the laboratory?
3. A solution containing 0.118 grm. of a dicarboxylic acid was exactly neutralized by 20 ml. of decinormal sodium hydroxide solution. Given that the acid as weighed was free from water of crystallization and contained only carbon, hydrogen, and oxygen, write out two structural formulæ consistent with the data supplied. Further, selecting ONE of the two acids represented, show how it might be synthesized. (H = 1, C = 12, O = 16, Na = 23).
4. Describe reactions which would allow you to distinguish (a) between propaldehyde and acetone; (b) between propyl alcohol and *iso*-propyl alcohol; and (c) between ethylamine and diethylamine. Give, in moderate detail, methods for the preparation of any TWO of the six compounds named.
5. Discuss the statement that the reactions of an organic compound depend on those of its constituent radicals, referring to a number of named examples.
6. Describe laboratory methods for the preparation of (a) acetylene and (b) ethylene. Discuss the properties of these two compounds.
7. How may acetic acid and oxalic acid be produced, starting from sugars? Give a general account of the properties of these acids.
8. Compare and contrast the properties of ethyl alcohol with those of glycerol; also, compare the esters of these alcohols.

PHYSICS

(Seven questions only to be answered. One at least must be taken from each section.)

Section A

1. Define *pressure*, *work* or *energy*, and *power*. Express each in appropriate metric units.
A steam engine has a cylinder 14 inches in diameter, and the piston has a stroke of 2 feet. The engine runs at 200 working

strokes per minute and the mean effective pressure is 100 lb. wt. *per sq. inch.* Calculate the indicated horse-power.

(1 horse-power = 33,000 ft. lb. wt. *per minute*; $\pi = 3\frac{1}{7}$.)

2. State Boyle's law. Describe an experiment you would make to test its truth, indicating clearly the actual measurements required. Sketch the general type of graph you would expect to obtain (a) if pressure is plotted against volume; (b) if pressure is plotted against the reciprocal of the volume.
3. What do you understand by osmotic pressure? How would you demonstrate its existence experimentally, and how do you account for it.

Section B

4. Explain how (a) a platinum wire; (b) a thermocouple, can be used for measuring temperature. Describe the apparatus you would need in each case. To what range of temperatures can each be applied?
5. What is Newton's law of cooling?

Explain carefully the different factors which determine the rate of cooling of a particular body.

In a closed copper calorimeter weighing 88 gm., 100 gm. of water cools from 55° C. to 45° C. in 300 seconds. In the same calorimeter, with the same surroundings, the same volume of oil (specific gravity 0.8) cools from 55° C. to 45° C. in 150 seconds. Calculate the specific heat of the oil.

(specific heat of copper = 0.1 calories *per gm. per degree C.*)

6. Define *relative humidity* and *dew point*.

How would you measure the relative humidity of the air by using EITHER (a) a wet and dry bulb hygrometer, OR (b) a dew point hygrometer. Describe the apparatus used and explain its principle.

Explain the formation of *dew*, *fog* and *hail*.

Section C

7. State the laws of reflection of light, and explain how you would test them experimentally.

A man standing in front of a vertical plane mirror wishes to see a full-length image of himself. What is the length of the smallest mirror which can be used and how must it be placed? Draw a diagram to illustrate your answer.

8. Describe the essential features of a spectroscope, explaining how you would use it to obtain the continuous spectrum of white light.

In what circumstances would you obtain

(a) a bright line spectrum; (b) a luminous spectrum with dark lines?

Give an explanation of the Fraunhofer lines in the solar spectrum.

9. Explain the terms "real" and "virtual" as applied to images and show with diagrams how each kind of image can be obtained with a convex lens.

What is the focal length of the projection lens of a lantern which will project a slide 10 cm. by 10 cm. as a picture 3 metres square? The screen is at a distance of 10 metres from the lens.

Section D

10. Show diagrammatically how resistances can be arranged (a) in series; (b) in parallel. What is the effective resistance of each arrangement?

Find the resistance of the wire which must be joined in parallel with a resistance of 12.4 ohms, in order that the combined resistance may be 12.0 ohms.

Describe briefly how you would measure a resistance by the Wheatstone bridge method.

11. Describe some form of tangent galvanometer and explain how it may be used to measure a current.

A tangent galvanometer has 20 turns of wire of mean radius 10 cm. Calculate the current in amperes which will give a deflection of 45° to the needle, the plane of the coil being in the meridian.

($H = 0.18$ dynes *per unit pole.*)

12. Describe the construction and principle of a simple type of dynamo, and show how it can be arranged to give (a) alternating current; (b) direct current.

Explain the advantages of using alternating current for the transmission of electricity in the grid system.

SECOND ASSOCIATESHIP EXAMINATION

PLANT BIOLOGY

(Six questions only to be attempted. One at least must be taken from each section. Answers should be illustrated by drawings and diagrams wherever possible.)

A

1. Discuss the statement: "Transpiration is an unavoidable evil."
2. What mineral elements, and in what form, does a green plant obtain from the soil? Indicate briefly the function of these elements in the plant.
3. Explain the processes involved in the disappearance of starch from a leaf in darkness and its reappearance as storage starch in an underground rhizome.

B.

4. What do you understand by *rusts* and *smuts*? Give a general account of any one disease of barley caused by either type of organism.
5. Explain the principles involved in the methods commonly used for freeing natural products from undesirable micro-organisms.
6. Compare and contrast the structure and reproduction of *Eurotium* (*Aspergillus*) and *Penicillium*.

C.

7. Explain how you would distinguish between the young stem and root of a dicotyledon as seen in transverse section under the microscope.
8. Give an account of the structure of the inflorescence of the barley. Indicate which of the features you mention are characteristic of the barley, and which of the grasses as a whole.
9. Explain the importance of chromosomes in the study of heredity.

ORGANIC CHEMISTRY

(Five questions only to be attempted.)

1. Discuss the significance of iso-electric point in relation to protein properties.
2. Explain the meaning of the term "diazotization," and discuss the general properties of the product of such action.
3. Describe several tests which would enable you to distinguish between glucose and fructose. Similarly, how would you distinguish between glucose and arabinose and between glucose and maltose?
4. Discuss the evidence in favour of the view that starch is composed of amylose and amylopectin. On what basis are these components defined?
5. Give an account of those properties of amino-acids which (a) permit their quantitative determination; and (b) allow the separation of certain amino-acids from mixtures.
6. Starting from benzene, draw up schemes for the production of the following: (a) acetanilide; (b) benzenesulphonamide; (c) phenyl-methyl ether (anisole). The reagents employed and the general conditions of working should be briefly indicated.
7. Discuss the use of ethyl acetoacetate in organic syntheses.
8. Write an essay on ONE of the following subjects:
 - (a) The chemistry of the plant cell-wall.

- (b) Methods of investigating the structure of disaccharides.
- (c) Modern views on the structure of proteins.

PLANT BIOCHEMISTRY

(Only six questions to be attempted.)

1. A good sample of British barley and a poor sample of Californian barley would yield distinctly different extracts as malt. What biochemical reasons explain such variations?
2. What vitamins are found in cereals and what changes occur during malting in the amounts present?
3. Outline methods for the measurement of the antiseptic power of hops by means of bacteria and by chemical methods. What changes are undergone by the antiseptic substances during brewing?
4. A sample of brewers' invert sugar is known to contain invert sugar, cane sugar and 0.80 per cent. ash (on sample). A 10 per cent. solution (w/v) has a specific gravity of 1.032.3° and gives a polarimeter reading of - 1.35° in a 100 mm. tube. After inversion with acid the reading (on half concentration) becomes - 0.81°.

Given—

- (i) $[\alpha]_D$ of cane sugar = + 66.5°.
- (ii) $[\alpha]_D$ of invert sugar = - 19.8°.
- (iii) Solution factor of the sugars is 3.88.
- (iv) Solution factor of ash solids is 8.0.

Calculate—

- (i) Total sugar solids.
- (ii) Per cent. cane sugar on sugar solids.
- (iii) Per cent. sugar on sugar solids.
5. What is the chief form in which phosphorus occurs in barley and what products are formed from it during malting, mashing and boiling?
6. In addition to alcohol and carbon dioxide what products are formed in smaller amounts during fermentation and how do they originate?
7. Discuss the concept of temperature optima in enzyme actions and illustrate from the enzymes concerned in the mashing process.
8. Give an account of the proteins of barley and of the transformations undergone in malting and brewing.

DIPLOMA MEMBERSHIP EXAMINATION

MALTING

(Answers to all questions should be attempted.)

1. Compare the advantages and disadvantages from the growers' and the maltsters' point

of view of:—(a) Spratt Archer Barley, and (b) Kenia Barley.

2. Describe either:—(a) An aerated bin with the necessary equipment for barley storage; or (b) A barley drying plant.
3. Describe in detail the process of making one of the following:—(a) Crystal Malt; or (b) Brown Malt; or (c) Black Malt.
4. Describe one of the following:—(a) A Galland-Henning Drum; or (b) A Saladin Box; or (c) A Swedish Box Drum.
5. The Standard Methods of Malt Analysis have recently been revised and a new factor recommended for calculating brewers' extract. What was the reason for the change, and what is the new factor?

BREWING

(Six questions only to be attempted. Answers should be as brief as possible.)

1. What precautions should be adopted in a Malt Room? Describe the use and maintenance of a modern malt mill. State how you would arrange your grinding programme, and settle the fineness of grind.
2. What points should your chemist and your engineer examine as a routine matter, and report upon, in connection with your supply of:—(a) brewing water; (b) refrigerating water; (c) attemperating water; (d) washing water? In what ways may one type of water be used for other purposes?
3. Discuss the propagation, maintenance, storage, and selection of your pitching yeast.
4. When, and how, would you clean your wort and ale mains, cocks and sluices. Are there any special points in design and erection, that you would insist upon, in a new installation?
5. Describe the racking and despatch priming procedure which you would adopt, and state what considerations would cause you to vary it throughout the year.
6. Discuss racking loss and its diminution.
7. Make out suitable hop grists, in percentages, for June brewings of running Mild Ales and Pale Ales. Include a suitable quantity of a New Variety hop, and state which one you would select. How would you use the hops, assuming both brews to be a first copper followed by a second?
8. A few cases of fining difficulty are reported from your houses. How would you investigate and remedy the trouble?

BREWERY AND PUBLIC-HOUSE CELLAR MANAGEMENT AND COOPERAGE

(Seven questions to be attempted. Two at least should be taken from Section B. Answers should be as brief as possible.)

A.

1. What brewery cellar conditions do you consider necessary for:—
(a) A primed running Mild Ale? (b) A draught Pale Ale? (c) A chilled, filtered and carbonated Pale Ale for bottle?
2. Priming, finings, and dry hops are usually added in the cellars. Which beers generally receive these additions, at what rates, and for what purpose?
3. A brewery has a small, slow, sale for a full, sweet, draught stout and has decided to bulk pasteurize this beer. Describe the necessary plant suitable for this process.

B.

4. What are the principal causes of beer infection in a public house? State what steps you would take to control them.
5. What method would you employ for (a) the cleaning of beer pipes in the public-house cellars; (b) washing and sterilization of glasses?
6. Describe a modern licensed premises from counter to cellar, including all necessary services in the handling of beer and its attendant problems.

C.

7. (a) State the period of time necessary to season naturally fresh-cut timber before making brewers' casks.
(b) Describe the method of stacking.
8. On examining the end of a stave, medullary rays are to be seen. Show these by a drawing and state why timber for brewers' casks is cut in such a manner.
9. In the event of a cask becoming slightly foul, what steps would you take to make it sweet again?

BOTTLING

(Seven questions only to be attempted.)

1. What do you understand by "chillproofing" and what is the action of the agents employed?
2. Describe in detail a method for the carbonation of beer. What are the laws governing the absorption of CO₂ by beer?
3. Describe any process with which you are familiar for "roughing out" beers prior to final filtration by pulp or sheet.

4. What is the cause of "bloom" on bottles leaving the washer, and what steps would you take to remedy it?
5. Explain in detail the principles underlying the operation of any modern automatic filling machine with which you are acquainted.
6. The shelf life of your pasteurized bright beers is not satisfactory. Where would you look for the possible cause, and what action would you take?
7. What is understood by, and what are the factors affecting, head formation and head retention?
8. A new bottling stores is being planned. What are the main principles you would wish to see adopted in its layout?
9. Discuss the relative merits of wooden cases and metal crates as bottled-beer containers.
10. What routine records would you expect to keep to enable you to determine, from month to month, your bottling costs and profits?
11. The palletization of goods for handling and storage has been much publicized. Discuss its possible application in the bottling stores.

ENGINEERING, INCLUDING FUEL AND POWER

*(All questions to be attempted.)**Boiler House and Steam*

1. State briefly the functions of a boiler feed-water economizer.
2. Explain why superheated steam is unsatisfactory as a heating medium in heat exchangers.

Fuel

3. How is the calorific value of coal determined?
4. What is the essential difference between bituminous coal and anthracite?

Power

5. What is meant by a "Combined power and heating system" when applied to a power and steam generating plant?

Refrigeration

6. Describe what is meant by direct expansion cooling.

Electricity

7. If a motor of 10 h.p. has an efficiency of 85 per cent., how many B.O.T. Units will it use when running at full load for 12 hours continuously?

General

8. Answer ONE of the following:—

- (a) Describe the principle and working of a jet condenser.
- (b) Compare a steam-heated boiling copper with a coal-fired copper from a fuel efficiency point of view.

SCIENTIFIC CONTROL

(Answers to five questions should be attempted.)

1. Discuss the importance of nitrogen compounds in wort and beer with special reference to the different mashing procedures adopted in brewing various types of beer.
2. What importance do you attach to the various analyses which are normally carried out on a sample of malt? From these figures what can you deduce about the malting process?
3. Describe in detail the various organisms which can produce ropiness in beer.
4. What determines the resistance of a beer to infection by various bacteria? Discuss the relative importance of the various factors.
5. What tests would you apply to a well water to assess its suitability for use (a) in a brewery; and (b) in a public house? Indicate very briefly water treatments that might be necessary in various circumstances.
6. Describe in detail what experiments you would make in order to assess the suitability of a new variety of hops for use in your brewery.
7. Considerable importance is attached to the aeration of wort and beer at various stages in the process; give reasons for this.
8. Give the approximate composition of dried brewers' yeast and discuss its value as a foodstuff.

REPORTS OF THE EXAMINERS ON THE
JUNE (1949) EXAMINATIONS

(The following, from the Reports of Examiners in the individual subjects, is published for the information of Candidates)

FIRST ASSOCIATESHIP EXAMINATION

Chemistry (Inorganic and Physical)

Thirty-five candidates completed this paper. The standard reached in the answers was not high, the average mark being 43 per cent. Many of the scripts were very poor in English and in method of presentation.

The physical sections were very weak indeed, the average per cent. on the purely physical questions being only 35. The question on p_H was very badly done, a large proportion of the

candidates being unable to select indicators for every-day analytical titrations. Few candidates had proper knowledge of how to determine molecular weights of substances in solution and a surprising number chose to describe impracticable osmotic pressure methods. Muddled thinking was shown in eight answers in statements to the effect that dissociation of the solute leads to high apparent molecular weight. The question on solubility product was, remarkably, attempted by only three candidates, none being able to explain the application of this important principle to laboratory operations which all must have carried out. This shows the regrettable and wide-spread barrier in students' minds between the lecture-room and laboratory.

The Inorganic sections were better done in so far as factual answers were required. Thus, in question 1, knowledge of methods of extracting metals from their ores was fairly satisfactory, but discussion of the principles involved in the selection of these methods for particular metals was, in all cases, either trivial or missing. Although very largely a matter of common sense, it is not specifically printed in the textbooks, or given in most lecture courses. Again, in question 6, the application of the le Chatelier principle, which requires a little careful thought, was very poorly done. It would evidently be salutary to institute a separate paper in Physical Chemistry.

Organic Chemistry

In view of the general nature of the questions set in this paper, it is extremely disturbing to find that some 40 *per cent.* of the candidates failed to obtain a pass mark. It is clear that a proportion of the entrants should not have presented themselves; it must be clearly understood that, unless the elementary principles of organic chemistry can be grasped, it is impossible to proceed with any advantage to a study of biochemistry in its relationship to brewing. However, at the other end of the scale there were some answers of outstanding merit. Answers to questions 1 and 2 were in general lamentably poor, and the simple calculation of question 3, though it gained some good answers, was an unexpected stumbling-block to many. Question 4 gained the most consistently successful results and 5 led to a few thoughtful answers. Question 6 was done well by about half the candidates; the rest revealed more ingenuity than accurate knowledge. The two last questions were very disappointing; the compounds to be discussed in these questions are surely of sufficient importance to deserve better knowledge. Candidates would be well advised to read questions carefully before beginning their answers, and to understand that marks cannot be gained by the writing down of

irrelevant information, however accurate and interesting. The examination is not a test of memory, but of understanding.

Physics

Many good answers were again received to most of the questions and the majority of candidates were able to make a satisfactory attempt at the paper. The answers were generally well and briefly expressed. In question 2 a rather surprising number of candidates could not draw the shape of the graph showing the relation between pressure and volume for Boyle's Law. In question 5 few candidates were able to explain carefully all the different factors which determine the rate of cooling of a particular body. The numerical example in this question, although really simple, required thought, and few correct answers were given. Many good answers were given to question 10. Relatively few candidates explained clearly the advantages of using alternating current for the transmission of electricity in the grid system (question 12).

SECOND ASSOCIATESHIP EXAMINATION

Plant Biology

On the whole the standard, though rather lower than that of last year, was satisfactory. The following points are noteworthy:—

(1) Every student appeared to believe that the uptake of mineral salts by the roots of a plant was dependent on the uptake of water, and therefore on transpiration. It should be strongly emphasized that the two processes are independent, and that the rate of uptake of mineral ions is not known to be significantly affected by the rate of uptake of water.

(2) Diagrams are appalling, with a few honourable exceptions. Most candidates have still to learn that a scrappy inaccurate and largely illegible diagram is worse than none at all. In an anatomical question, any attempt to fill in details of cells where only tissues are concerned, is misguided; a good "low-power diagram" is invariably preferable.

(3) Minor points noted were: (a) a tendency to state that mineral salts and sugars move from cell to cell "by osmosis"; (b) no realization that there is any problem involved in the transport of sugar through the phloem; (c) several candidates stated that rusts and smuts are confined to the cereals.

Organic Chemistry

The standard reached was definitely higher than that of the First Organic paper. Nevertheless, it is surprising—and somewhat disappointing—that best results were obtained with what might be called the "purely organic" questions (Nos. 2, 6 and 7), the answers to which

were frequently very good indeed. Question 1, on protein properties, was also well done, but the favourite "structure of proteins" in question 8 was very poor. It was something of a shock to find how little many of the candidates knew of the properties of sugars and amino-acids, and attempts to discuss the structure of starch usually provided a curious mixture of ancient and modern knowledge. Protracted irrelevancies were a feature of many of the answers, and it is again apparent that a principal difficulty of many candidates is not an acquiring of facts, but of acquiring an understanding of the relation of the facts to one another. If they possessed some facility for selecting the relevant facts from the mass of information which is undoubtedly available in most cases, and if they could appreciate the logical relationships of these facts, many of the candidates would have received far higher marks.

Plant Biochemistry

This year, all the candidates appeared to have obtained their knowledge from reliable modern sources. Although in some cases the studies appeared inadequate, the majority were adequate to good. None of the candidates answering question 1 appeared to appreciate the biochemical reason for varietal differences in extract yield between British and Californian barleys. In most cases, the numerical example was not approached on the right lines or calculated correctly.

DIPLOMA MEMBERSHIP EXAMINATION

Malting

With one exception the standard can be considered high. The only point which calls for

comment was the average lack of knowledge on the subject of the *Standard Methods of Malt Analysis*.

Brewing

Many candidates did not show sufficient appreciation of the importance of detail in the application of their theoretical brewing knowledge to practice. This was particularly so in regard to the questions on cleaning and racking loss, and to some extent that on yeast. The questions on malt, water and yeast (except as above) were in general dealt with well, though some candidates did not use a microscope in the selection of their yeast. The question on hops was, in the main, avoided.

Brewery and Public-house Cellar Management and Cooperage

On the whole, these papers were good.

Bottling

It was noticeable in general that the questions on the theory of the subjects were much better answered than those dealing with the practice of bottling.

Scientific Control

Answers to questions in this examination were, on the whole, reasonably satisfactory. The change which was made in the form of the Examination Paper set, namely, asking for answers to five questions out of eight instead of asking for answers to eight questions, was reflected in the much higher standard of the answers, and appears to have been completely justified.

MEETING OF THE LONDON SECTION HELD AT THE HORSE SHOE HOTEL,
TOTTENHAM COURT ROAD, ON MONDAY, 11th APRIL, 1949

MR. B. M. BROWN in the Chair.

The following paper was read and discussed:—

**MALT ANALYSIS: A DISCUSSION OF THE RECENT REVISIONS
IN THE STANDARD METHODS OF THE INSTITUTE**

BY A. A. D. COMRIE, B.Sc., F.R.I.C.

The revised methods (this *Journ.*, 1948, 179) are critically discussed. The need is expressed for a statement of expected and permissible limits of variation in the results obtained for a given malt; the provision of a reference sample of malt, standardized by collaborative analysis and available to analysts throughout the country for checking purposes, would be of considerable help.

It should be pointed out that the comments and suggestions made in the course of this review are personal and not necessarily those of the Analysis Committee. To take the methods in the order in which they have been published (this *Journ.*, 1948, 179), *Pale Malts* come first.

Moisture.—Electrical heating is now recommended in place of gas heating for the oven. It is cleaner and not liable to the error (which can be considerable) which arises when gas fumes are not completely excluded from the oven. Some analysts probably wish to use a thermostatically-controlled electrically-heated air oven, and experience suggests that such ovens are satisfactory provided they are calibrated as to thermometer reading and position of the malts in the oven against a boiling-water oven. This might not be necessary if the oven were fan-circulated.

Since it is an essential of the boiling-water oven that the malts should be on the floor of the oven only, it may be suggested that a flat oven, opening at the top by a removable lid instead of by a door at the front, would be simpler and make better use of the oven space (Fig. 1). The air entering the oven at *A* is preheated by the boiling water and is spread over the base of the oven by a concave baffle, *B*, before escaping at the ventilators *C, C*, in the lid. The baffle is attached to the handle of a tray, *F*, on which the malt tins or dishes are set. There is an inset water gauge, *D*, and a filling orifice, *E*, to which a condenser may be attached. The oven can conveniently be made of welded copper with sheet asbestos fastened to the sides and top to conserve the heat, which is preferably produced by immersed electrical elements with a

three-heat switch (not shown in the diagram). An oven of this type with internal dimensions 13 × 11 in. would hold about 30 dishes.

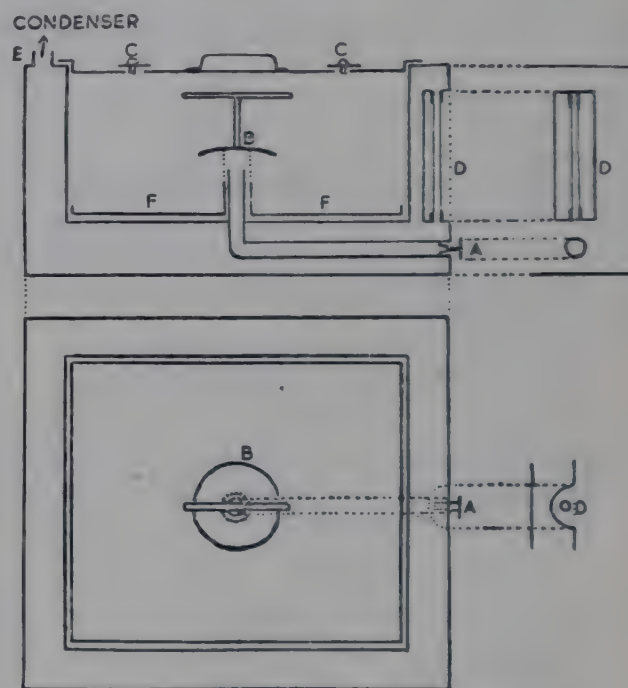


Fig. 1. Proposed malt moisture oven.

Extract.—There have been no changes in the actual technique, but in view of the introduction of the Permanently Soluble Nitrogen (P.S.N.) determination a word needs to be said about temperature control. A few experimental mashes made some years ago suggested that close temperature control, for English malts of fair quality, was not a critical factor as far as extract was concerned. Incidentally, some Californian and Indian six-row malts analysed at the same time were more susceptible to temperature variations. However, disregarding the possibility of

having imported malts to analyse again one day, if P.S.N. is going to be determined on the wort, accurate control of the mash temperature is essential. How this may best be secured is an open question; such things as asbestos-board screens to prevent draughts are obviously helpful. But is close temperature control of equal importance all through the mash period? Probably not. Recent work by I. A. Preece (see this *Journ.*, 1948, 335) has shown that most amylolytic action is over in the first 15 min. of the mash. The results of a test made on the fall of diastatic power and change in opticity of a brewery pale-ale mash illustrate this (see Table I). The initial high rate of destruction of the diastase, and the comparatively small change in the opticity after the initial stage of the mash, together show that the composition of the wort is largely determined in the early part of the mash. Since we are working at a temperature near the inactivation point of malt proteases it is probable that most of the proteolytic action is also over early in the

TABLE I

FALL OF D.P. AND OPTICITY IN THE COURSE OF A BREWERY PALE-ALE MASH

Time (minutes).	D.P., ° L. of malt	Opticity ($[\alpha]_{D^{580}}$) of wort.
0	28	(180)*
10	7	123
27	4.5	120
37	3	120
54	2.5	119.5
70	2.5	118
82	2	116
98	2	114
112	2	113
132	2	114

* This figure is hypothetical.

The procedure followed in this test was to take four samples each time from different parts of the tun, mix them, and strain through muslin to remove the grains. About 250 ml. were brought rapidly to the boil (for opticity) and another 250 ml. cooled rapidly in ice (for diastatic power). The time of each sample was taken when this had been done (first column). The sp. gr. of the wort was determined and the opticity of the boiled portion found in the usual way. The diastatic power was determined by making starch conversions with 5 ml. of the "10 min." and "27 min." worts, and with 10 ml. in the case of the remainder, the converted solutions from the 10 ml. conversions being diluted 25 to 50 ml. for titration. Blank titrations against 5 ml. Fehling's solution were made on 5 ml. of each wort diluted to 200 ml. Then, if *A* is the conversion titration and *B* is the blank titration, the true D.P. titration is $AB/(B-A)$, and the diastatic power of the malt in the mash (assuming a sp. gr. of 1014° as equivalent to a 5 per cent. malt mash) is given by the expression $5,600 (B-A)/ABC$, where *C* is the sp. gr. of the wort in excess of 1,000. (The spec. grav. of the mash is used here on the assumption that extraction of diastase from the malt will keep pace with extraction of total solids from the malt. The total diastase is the figure required, and if the sp. gr. is ignored the diastase still in the malt is ignored). The initial D.P. was calculated from the composition of the grist.

mash time. This would mean that temperature control is less critical after, say, the first 15 min. If it appears that this point is being laboured, it is because it seems well worth settling, for on its outcome would depend the type of temperature control system needed. Fifteen minutes of accurate control could be maintained by hand, but an hour or two would require elaborate thermostatic and stirring devices.

What is most important is that the initial temperature of the mash should not exceed 150° F. and here flasks appear to have an advantage over beakers. In a recent communication containing observations on malt analysis technique (*ibid.*, 1948, 320), H. E. Kelly and T. S. Bremner, using beakers, found that local overheating during the mashing-in caused a loss of about 0.5 lb. of extract in some cases. With flasks it is possible to get more intimate and immediate mixing than with beakers, and so avoid the overheating. The importance of thorough mixing of the mash after making up to volume is stressed. One reason for this is the slowness of diffusion of the relatively stronger wort from the particles into the added water, and another reason is the surprising difficulty of getting efficient mixing in a flask with a small free air space. A personal preference is to transfer the mash after making up to volume to a 1.5 litre flask for the final mix. The volume is made up at 68° F., in conformity with the standards now in force for volumetric glassware. Tint is to be determined on a final 25 ml. of the filtrate in order to reduce to a minimum any interference due to haze in the wort.

There follows an important change from the quantitative point of view, and that is the replacement of the factor $\times 3.36$ by the factor $\times 3.565 - 4.8$ for the conversion of specific gravity into extract *per 336 lb.* It has long been recognized that the arbitrary standard of 15 ml. as the volume of the grains from 50 grm. of malt is incorrect for most English malts. As far back as 1905 Ford and Guthrie pointed out that the volume of the grains from an average English malt was nearer 10 ml. than 15 ml., and this has been confirmed by the more recent work of B. M. Brown (*ibid.*, 1943, 195), J. R. Gwilt (*ibid.*, 1947, 152), and L. R. Bishop (*ibid.*, 1944, 139). But whatever volume was adopted, so long as it was a fixed one it would be accurate only for malts giving a certain

extract, because grains volume is inversely proportional to extract. The adoption of a new fixed value for grains volume would have a further disadvantage; it would mean the scrapping or recalibration of all the 515 ml. flasks in use. The Analysis Committee therefore decided to adopt the more accurate and simpler method of continuing to use the standard 515 ml. flask, but using a new factor for the calculation of the extract. This factor takes account of the inverse relationship between grains volume and extract, and is based on the investigations of Bishop. It has been carefully checked, theoretically as well as practically, by Bishop and his colleagues (*ibid.*, 1948, 189, 194). The effect is to raise the extract of the average English malt as determined by about 1 lb. In malts of exceptional quality the increase will be more than 1 lb., and conversely it will be less in malts of poor quality and in malts from six-row barleys.

Cold Water Extract.—The revised method lays emphasis on keeping the temperature at 70° F. during extraction. There are no changes in procedure, and in regard to other conditions Kelly and Bremner (*loc. cit.*) have found that considerable latitude is permissible except for one thing. It is essential that not less than the stipulated concentration of ammonia be present in the water used for extraction.

Diastatic Power.—Here, again, the actual determination is the same as it was before, but the Fehling's solution A is now standardized at the strength used by Lane and Eynon in compiling their sugar tables. This is an advantage, for the one solution is now available for both D.P. determinations and sugar analysis. Previously, the method of standardization whereby 5 ml. of Fehling's solution had to be equivalent to 25 ml. of 0.1 per cent. invert sugar solution, meant that the Fehling's solution was slightly weaker than that demanded by the sugar tables, and the chemist who wished to determine the copper-reducing power of a sugar had to prepare a fresh Fehling's solution for this purpose. D.P. values are about 4 per cent. less with the new standard than with the old, a change which is hardly significant in D.P. work.

It is important to keep the 200 ml. flasks used for the conversions exclusively for this purpose if possible. Some time ago, trouble

with some D.P. determinations was traced to one of the 200 ml. flasks. It was discovered on enquiry that this flask had accidentally been used for the preparation of some decinormal silver nitrate solution, and sufficient of the silver ions remained adsorbed on the glass, even after thorough washing, to inhibit almost entirely the action of the diastase. Zinc, copper, chromium, cobalt and mercuric ions have been tested for this oligodynamic effect; the only metal which had a potency approaching that of silver was the mercury, but copper had a slight action. Ordinary methods of cleaning the flasks in these cases were useless; cyanide had to be used.

Nitrogen.—Determinations of Total and Permanently Soluble Nitrogen have been included in the *Standard Methods* for the first time in view of the importance that has come to be attached to them in recent years; the P.S.N. as an indication of yeast feeding quality, and the ratio P.S.N./Total N. as the coefficient or index of modification—a ratio which it may be preferred to call the protein index. The Kjeldahl procedure prescribed is so well known as to need no comment. The importance of close control of the mash temperature where P.S.N. is to be determined on the wort has already been discussed. The method of eliminating coagulable nitrogen is, of course, arbitrary, but should give comparable and reproducible results. In passing, in view of the recommendation that the distillation should be carried out with the connecting tube sloping up from splash-head to condenser, it is a pity that the manufacturers of splash-heads seem invariably to make their side tubes slope the opposite way.

Statement of Results could possibly be followed, when sufficient experience of the methods is available, by "Permissible Limits of Variation," expressed, *e.g.*, as "Standard Deviations," to avoid argument over a difference which may not be significant. These limits could be made applicable to sample or to bulk, which are two very different things, of course, for the second involves not only errors of analysis, but errors of sampling. From the results of replicate analyses made from time to time (but not from any calculation of standard error), replicate analyses on the same sample might be expected to show no greater variation than 0.2 per cent. for moisture, 0.5 lb. for

extract, 0.5 unit for tint, 0.5 *per cent.* for cold-water extract, and 1° for diastatic power.

Extract of Coloured Malts and Malt Adjuncts.—These two types of material are being considered together because the mashing procedure has been modified in the same way for both. The new method is simpler and gives more consistent results than the earlier method. Shortly after the publication of the Standard Methods of 1933 it was noticed that the preheating of the cold malt extract to 140° F. before adding it to the mash reduced its amylolytic power. This partial destruction of the amylase, coupled with the considerable time it took for the mash to steady itself at 150° F., caused erratic results for the extract. Moreover, when flaked barley came along, mashing it with hot water before the addition of the cold malt extract gave rise to troublesome "balling." If the cold malt extract is added to the grist before adding the mashing liquor, these troubles are avoided and better replicate results are obtainable. The figures in the first two columns of Table II show the results of a number of

is a danger of getting low extract figures from coloured malts if the D.P. of the malt used for the cold malt extract is much below 40°.

Much time can be saved by using a diastatic malt syrup instead of the cold malt extract. The syrup should be pale in colour and have a D.P. of at least 60°; a solution of 20 *per cent.* or less will then be strong enough for the conversion. This is not a specified method, and for some reasons it gives results with coloured malts that are up to 0.5 *lb.* lower than those given by the use of freshly prepared cold malt extract (see the figures in the third column of Table II), but this comparatively small difference is more than offset by the greater ease of operation. One word of warning. When testing the suitability of a malt syrup for this purpose, it is advisable not to rely solely on a determination of its D.P., but to make one or two comparative mashes also. I have known the mash-converting power of a malt syrup to diminish with age, although its D.P. Lintner showed very little change.

One other point is worthy of mention. To get an initial temperature of 150° F. by the new procedure, the temperature of the mashing liquor has to be about 190° F., and the risk of local overheating that was mentioned earlier is increased. Using flasks, I have made comparative mashes with flaked barley and two kinds of coloured malt, one mash of each pair being made with water at 190° F. and the other preheated in the mash bath before being mashed with water at 158° F. There were no significant differences between the results obtained by the two ways.

It will be noticed that, as with pale malts, the factor for converting specific gravity to extract has been altered to make the more correct allowance for grains volume.

Having come to the end of this survey of the changes in the *Standard Methods*, there are one or two final remarks of a general nature which seem desirable. Mention has already been made of the value of limits of variation for each determination. Any truly complete method of analysis should, after laying down the procedure, give some idea of the experimental errors inherent in the method, and what effect a departure from standard procedure at any point is going to have. The analyst would then know where he had to be particularly careful and where he could ease up; possibly where he could expedite the analysis if necessary with least

TABLE II

EXTRACTS OF COLOURED MALTS AND MALT ADJUNCTS
in *lb. per 336 lb.*

Sample.	Old method (1933).	New method (1948).	Using diastatic malt syrup.
Brown malts ..	83.7	85.2	84.6
	82.0	83.8	83.3
	84.4	85.9	85.4
Amber malts ..	86.3	87.1	86.5
	87.3	89.1	88.7
	87.9	89.0	88.5
Flaked barleys ..	83.9	84.5	84.8
	78.6	80.3	80.8
	86.7	87.8	87.3
	80.3	82.0	82.1
	83.7	85.2	84.6

mashes of coloured malts and flaked barleys made by the old (1933) and the new (1948) procedures. It can be seen that the extract figures given by the new method are between 1 and 2 *lb.* higher than those given by the old method.

The standard method specifies a malt of D.P. 30° to 40° for the preparation of the cold malt extract. Experience suggests that there

loss of accuracy. An illustration of this occurs in the communication by Kelly and Bremner (*loc. cit.*) where it is shown to be more accurate to use the cold-water extract solution for the determination of diastatic power than it is to do the reverse. The analysis committee would probably like to know whether the methods are thought needlessly detailed or not detailed enough, and whether the field of application of standard methods in brewery work can be profitably enlarged, and if so, in what direction.

Naturally, the existing methods are not accepted as final. To quote from a recent report of the Nutrition Committee of the Food and Agriculture Organization of the United Nations:

"Standardization of methods of taking and preparing samples, and the development of specific analytical techniques generally, require extensive collaboration and continuous review and modification."

The analysis committee exists to provide that continuous review and modification, but

possibly something more could be done in arranging collaborative tests. Many will remember a study of this sort arranged by Prof. Hopkins, of Birmingham University, in 1938, when some of the results were disconcerting. When the Institute's research centre is in operation it is anticipated that a study of methods of analysis will be one of its activities. In the meantime, it may be suggested that a "reference malt" should be made available at some centre so that any analyst could check his procedure if he wished. The malt would be kept in airtight containers holding about 500 grm., would be renewed periodically, and would need to be standardized by collaborative analysis. In that way not only would a useful service be provided for analysts, but a body of data would be acquired as to the effectiveness of the *Standard Methods*.

The Laboratory,
Dallam Brewery,
Warrington.

MEETING OF THE LONDON SECTION HELD AT THE HORSE SHOE HOTEL, TOTTENHAM COURT ROAD, ON MONDAY, 10th JANUARY, 1949

Mr. B. M. BROWN in the Chair.

The following paper was read and discussed:—

FAMOUS BREWERIES. 4.* PARK ROYAL BREWERY

(Messrs. Arthur Guinness, Son & Co., Ltd., London.)

By M. W. PLUMPTON, M.I.Mech.E., M.Inst.F.

An account of the plant available is given in some detail, its mode of operation being outlined with individual departments discussed independently. Attention is given to problems of sterilization of plant and of fuel economy.

THE construction of Park Royal was an event in the history of English brewing in that it was the first time in this country that a large brewery had been built on a clear site—one might even say, a green field—with nearly 200 years' experience of an existing brewery to draw upon and the difficulty overcome of starting up a completely new plant for a process which depends so much upon carefully conditioned vessels and other equipment. The operations of the Company and the

Dublin Brewery are well known to the Brewing Industry, and as for some years it had been apparent that, since so large a proportion of the output from Dublin was being sent to England it would be economical to establish a brewery here for that trade, in 1933 it was decided to do so. Once the capacity of the English brewery had been determined by considerations of trade and distribution, the design of it was commenced and at the same time a survey made of probable sites.

The site selected was convenient to the

* For Parts 1, 2 and 3 see this *Journ.*, 1943, 1, 105, 265.

Great Western Railway (now British Railways, Western Region) and the Grand Union Canal, and had excellent road connections, being adjacent to two of the main arterial roads in north-west London. The site was planned with a view to developing it on modern lines not only from the point of view of factory operational efficiency, but also to provide those amenities and facilities for the staff which have always been a feature of the Company's activities. The area of the site selected was about 137 acres, the eastern portion of about 45 acres being allocated to the brewery proper and the remainder of the land north and west to playing fields, employees' houses and other amenities. A feature of the site is the fall of the land contour from south to north, and the main brewery buildings were arranged with a view to the maximum use being made of this fall, the process flow following it as far as possible.

The brewery was designed for a normal production of 650,000 barrels *per annum*, with an overload production capacity of just over 1,000,000 barrels *per annum* with all plant in operation. The wisdom of this latter decision was manifest during the War, when with so many difficulties affecting materials, fuel, and transport from Eire, the ability of the Park Royal plant to produce this additional output was invaluable. Construction was commenced in November, 1933, and the first trial brew was run through in February, 1936. By the time War broke out in 1939 production had begun to approach the design figure as more and more plant was conditioned and brought into operation.

The buildings are generally designed and constructed in five large monolithic blocks on a line running roughly north to south, the front of the buildings presenting an attractive prospect from Western Avenue. The main buildings are supported on reinforced concrete piles 14 in. square varying in length from 35 ft. to 45 ft., each pile carrying a load of about 45 tons. The plant and machinery are carried on a braced steel-framed structure on the outside of which is a second framing anchored to the main frame supports and carrying the wall and brickwork panelling.

Water system.—The water distribution system is controlled from an intake and meter house through two 12-in. mains with two meters on each main, either meter being in use at any one time, the other being in reserve to ensure continuity of recording. On

leaving the intake meter house the intake mains divide to form a ring main round the whole brewery, secondary feeder mains being tapped off to each department and the control valves being so arranged that full water requirements can be fed through either side of the ring main. The intake system is designed for an ultimate capacity of $1\frac{1}{2}$ million gallons *per day*, and the present daily average maximum is about 750,000 gallons.

The heaviest user in the brewery is naturally the refrigerating plant, which in the summer months accounts for a maximum demand of up to 40,000 gallons *per hour* for the condenser cooling water. This water leaves the refrigerating plant condensers at about 85° F. and is discharged to overhead receivers in the brewhouse liquor system, any excess to holding capacity overflowing to two compounding reservoirs each of 250,000 gallons storage capacity. The reservoir water is pumped through a secondary ring main of 12 in. bore round the brewery, supplying Power Station services such as turbine condenser cooling water, oil cooler cooling water, boiler make-up and general rough washing services throughout the brewery. In terms of the ratio water/beer, the brewery consumption is of the order of 6 : 1 in summer, and 5 : 1 in winter.

From the point of view of continuity of supply the system has proved most satisfactory in use, but in view of present-day trends in water conservation and fuel economy, the possibilities and economies of using forced-draught water-cooling towers for the refrigerating plant condenser cooling water and the steam turbine condenser cooling water are being considered. With a forced-draught cooling tower the cooling water could be re-circulated over the tower, thus saving the continuous intake at present necessary.

The malt store.—As constructed, the malt store can hold some 125,000 quarters, using both the main and auxiliary silos, the latter being in the inter-spaces between the main circular silos.

The malt store building is about 100 ft. high, the silos being of reinforced concrete masked by brickwork panelling. There are 127 silos each 12 ft. diameter by 65 ft. deep and holding approximately 760 quarters each, and there are 105 inter-space silos, with capacity varying from 130 to 165 quarters. Particular interest attaches to the construction of the reinforced concrete silos in the malt

store, as they were among the first to be built in this country on the moving form principle with the concrete being continuously poured. The complete structure was erected in 13 days, the average speed of the erection being 5 ft. *per* day. In this method of construction, timber formers are built to the size and shape of the silos and lifted slowly by jacks as the concrete is continuously poured. It ensures not only rapidity of erection, but also a continuous smooth surface free from any joints which might arise from different setting consistencies of concrete inevitable in the fixed shuttering and batch pouring system.

The intake system had to be designed to receive by road or rail five different grades of malt simultaneously from different sources of supply, and to keep them separate through the screens and ultimately to the storage silos. To allow of this being done, the intake system was arranged in five separate units, each having a capacity of 250 quarters *per* hour and consisting of:—intake elevators; weighing machines before and after screening; magnetic separators; wire cylinder screens for cleaning and grading; suction filter dust collecting plant; and delivery elevators. Malt can be taken in at any unit, to be cleaned, screened and weighed, after which it is delivered to any one of the storage silos by means of the bucket elevators and the belt conveyor system. The stored malt can be turned over or recleaned whenever necessary. Similarly, malt can be drawn from any silo, to be weighed and passed over to the brewhouse mills.

The wire-mesh screens are constructed of 18 s.w.g. wire, the spacings for the various screenings being as under:—

Passing	Wires <i>per</i> ft.	Space (in.)
Culms	108	0.063
Small and broken grains ..	86	0.095
Large grains	60	0.152

The whole system is automatically sequence controlled through a control board on which the operator plugs in the particular screening system he wishes to use and the inter-connecting elevators and belts. The two delivery belts from the malt store to the brewhouse have a capacity of about 200 quarters *per* hour each, which allows a day's malt to be transferred in three to four hours on one belt.

Briefly, the malt is received in sacks either by road or rail, emptied into the intake hoppers on the ground floor from which it is elevated to the receiver at the top of the system on the sixth floor and weighed. The bottom hopper of the weigher is arranged to divide the malt automatically into two equal streams which then pass over the magnetic separators to the malt screening cylinders below. Each intake unit has two banks of wire-mesh screens through which the grain passes in series.

The first bank is located on the fourth floor of the malt store and consists of twin screens separating out culms and large and small grains, the overtails being stones and large foreign material. The second bank arranged on the third floor separates out any culms which have passed the first screen and small and broken grains, the overtails being the large grains. It is necessary to separate the small grains which will pass an 86 mesh (0.091 in.) as they require a special setting of the mill rolls to give the required grist. Large malt is overtailed and passed through an aspirator connected to a suction fan which draws off light and diseased grain.

Large malt from the final screening section passes to a weigher and afterwards to an elevator which takes it to the top of the house and to the system of eight transfer conveyors arranged above the silos from where it is discharged from travelling carriages to any silo. The small grain plant removes any remaining culms after which the malt passes through an aspirator which draws off light and diseased grain. There are no overtails from this machine as all large impurities have already been removed. The thin malt from the aspirator is delivered by belt conveyor to two Carter disc separators which remove small round seeds, half grains, *etc.*, from the malt, sound malt passing to the weigher and elevator for delivery to silo. The malt is drawn off from the silo bottom through valves and chutes to a system of 16 cross-belt conveyors below, and thence to transfer conveyors and to large automatic weighing machines, elevators and transfer belts to the brew house.

The silos were completed in October, 1935, and by February, 1936, it was necessary to provide storage capacity at Park Royal for incoming malt. As this was the time of the year with high humidity and as malt cannot be stored in damp silos, some means of

quickly drying out these large circular silos had to be adopted. The conclusion reached at the end of various tests was that malt placed in a silo which had been heated would not absorb as much moisture as a non-heated silo if the silo was filled (and kept full) as soon as possible after drying had been stopped. To dry the silos artificially, large electric heaters of 10 kw. capacity were lowered into them and left there for 10 days, the heaters being raised gradually through the full height of the silo over the period. The outlet valve at the bottom was opened and grids were placed over the inlet opening at the top so that a current of dry, warm air was passing through the silos continuously.

The Hop Stores.—There are six hop stores with a total storage capacity of 950 tons. Each is a steel-framed, brick-pannelled building, the steel staunchions being encased in concrete with chamfered corners to prevent damage to the hop bales, the floors of the stores being covered in hard-wood blocks laid on the concrete. These stores were designed to provide conditions necessary for satisfactory storage of hops, *i.e.* cool and dark buildings with a minimum movement of air. Each store is about 23 ft. high by 130 ft. long by 60 ft. wide, and each floor is designed for a load of $2\frac{1}{2}$ cwt. *per sq. ft.*

The hop bales, handled into and out of the store by bale elevators, are stacked up to 18 ft. high with 18-in. walk-ways between, which brings them within about 2 ft. of the top of the store. Stacking is carried out by means of an "iron man," an electrically-operated lifting jib on a travelling platform. Although this has proved satisfactory, in future we would probably be favourably inclined towards an overhead travelling crane with the lifting carriage "nested" inside the crane frame to reduce head clearance.

Experience up to the time of designing Park Royal indicated that there was no marked economic advantage in artificially cooled hop storage, as the risk of possible deterioration of the hops due to temperature rise in a hot summer was more than outweighed by the heavy capital cost of the cooling plant. Consideration of the problem to-day and the relative costs might well decide in favour of the artificially cooled store.

The Brew House.—The brew house, designed for a nominal output of 3,000 barrels

per day, is arranged on the infusion method of mashing, the main plant consisting of six mash tuns arranged in line on the south side of the brew house, with four boiling coppers in line on the north side. Each brew requires about 20 hours to complete from the commencement of the mash to the running of the last worts to the fermenting house, which permits of only one brew *per day*.

Normally, the six mash tuns are worked five days out seven, leaving two days for maintenance and adjustments, although during the war, they were worked 13 days out of 14 over long periods. This resulted in a heavy deferred maintenance programme to be dealt with after the war and which is only now nearing completion.

Before dealing with the plant in detail, perhaps a brief description of the operations in the brewhouse will be of interest. Malt is transferred from the malt store by belt and delivered into a chain-link conveyor running above the six mash tuns. Each mash tun is a unit complete with its own weighing machine, whole malt hopper, malt mill, grist hopper and Steele's masher. The chain-link conveyor delivers the malt to the automatic weighing machine, the malt passing over a magnetic separator for removing pieces of iron and steel. From the automatic weigher, the malt is fed into a malt hopper large enough to take the requirements for one day's brew for one mash tun. Below the malt hopper is arranged the malt mill delivering the ground malt into the grist hopper below. The grist is fed into a Steele's masher, where it is mixed with the mashing liquor before being fed into the mash tun.

The worts are run off from the mash tun from underneath the false bottom plate and delivered to the underback from where wort pumps deliver it either to the coppers, or if no copper is available, to the upperbacks. After the addition of hops and the usual boiling off in the copper, it is struck off into the hop back from where the worts are pumped up to wort coolers arranged at the top of the building. After the usual standing time, the worts are run down to the fermenting house.

The six malt mills are of the two-high four-roller type, each having a capacity of 30 quarters *per hour*. Each of the six mash tuns, designed for a mash of 110 quarters, is a circular cast-iron unit 20 ft. in diameter and about 7 ft. deep, the lower portion of the tun

being insulated with plastic magnesia $2\frac{1}{2}$ in. thick. The mash tun covers are of copper with the usual balance-weight lifting gear. The false bottom plates are of gunmetal with 22 s.w.g. slots, *i.e.* 0.028 in. wide. These plates are regularly cleaned about every four months with a hot caustic soda solution. The liquor space below the false bottom plates is $2\frac{1}{2}$ in. At present, the bottom of the mash tun is cleaned by lifting all false bottom plates which involves a considerable amount of labour, but the fitting of a pressure nozzle cleaning system is being considered, which will reduce the frequency of the laborious lifting and replacing of the false bottom plates from being a daily routine, to perhaps a weekly one, or longer. Each mash tun has its own independent geared drive unit driven by a 10 h.p. motor and provision is made for coupling up the drive units of adjacent mash tuns by means of an extension shaft should there be a prolonged failure on a driving motor during the mash. The sparge arms are driven through a unit gearbox.

There are four wort coppers each with a holding capacity of 650 barrels. They are designed for pressure boiling at about $1\frac{1}{2}$ lb. p.s.i.g., giving a wort boiling temperature of about 216° F. The coppers are steam heated, being fitted with a six-wing vertical tubular heater having a heating surface of 176 sq. ft. and a five-loop heating coil with a surface of 160 sq. ft. arranged at the bottom of the copper. Steam is supplied at a pressure of 100 p.s.i.g., being reduced steam from the high-pressure steam lines. Each copper heater unit is capable of heating up a full copper in $1\frac{1}{2}$ hours, and is capable of boiling off a maximum of 30 barrels *per* hour. Steam consumption is of the order of 9,000 lb. of steam *per* hour on the maximum duty. The copper is fitted with the usual pressure control and vacuum breaking and striking-off valves.

Originally the copper domes were bare copper in accordance with general brewery practice, but during the war the domes were lagged with $2\frac{1}{2}$ -in. magnesia with a saving in fuel of approximately 150 tons of coal *per* annum.

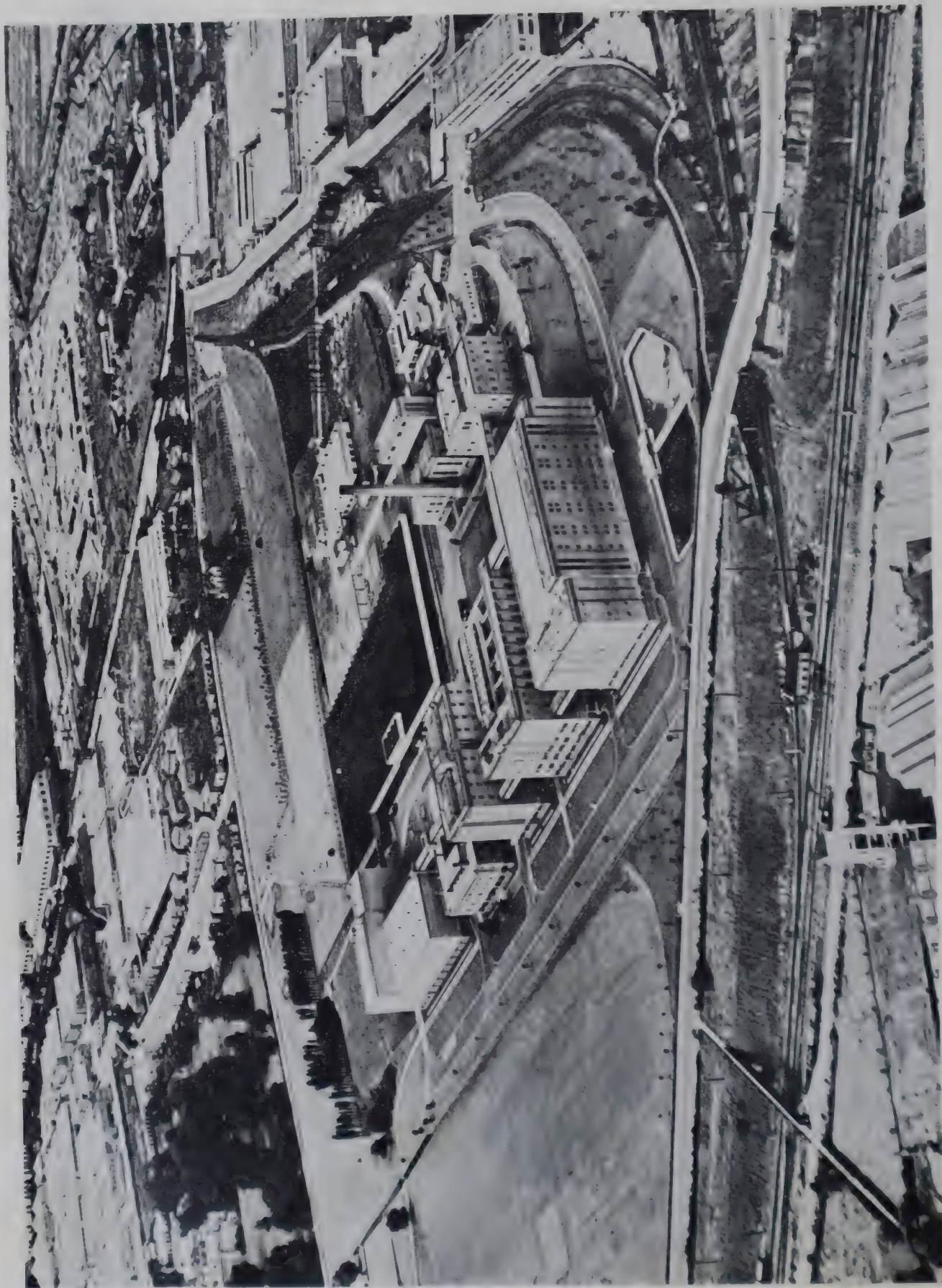
At the time Park Royal was being designed, the coppers in the Dublin brewery were coal fired with the exception of one which had been fitted with a steam heater for experimental purposes. From the brewers' point of view, brewer colleagues appear to be quite satisfied with the result of steam

heating—at least they have not yet asked for the steam heaters to be taken out in order to revert to coal or oil firing.

The vapour from boiling off is led through a lagged vapour main to jet condensers, specially designed for the purpose, and the cooling water is normally that returning from the wort refrigerators at a temperature of 130° F., which is just below the critical temperature for the deposition of the temporary salts. If, however, boiling off is taking place at a time when the wort refrigerators are not in use, direct cold water is used. In either case, the supply can be regulated automatically to give the condensate a temperature of 205° F., or the condensate re-circulated over the condenser to raise its temperature. The condensate is run down to the brewhouse low receivers for use in the brewhouse liquor system. Each copper has its unit condenser, which can deal with the maximum rate of reduction of 30 barrels *per* hour. The jet condenser is a simple and less costly plant than the more usual surface condenser, but it can only be used where a use can be made for the mixture of cooling water and condensed steam, *i.e.* distilled water. A brewery, of course, can make good use of such a supply of hot water.

The factors determining design are quantity and temperature of the cooling water available; the quantity and temperature of the steam or vapour to be condensed and the temperature of the condensate required, the latter being variable depending upon the amount of cooling water used or available. Conserving the heat of the copper reduction vapour in the way adopted saves approximately 750 tons of coal *per* annum.

There are two hop backs each of 800 barrels capacity of more or less orthodox design in that they are circular vessels of about 26 ft. 6 in. in diameter and 9 ft. deep, constructed in copper bearing steel to resist corrosion and the usual gun-metal false bottom plates, the slots being 19 s.w.g. *i.e.* 0.040 in. The hop backs have copper domes with a chimney taken up through the roof for disposing of the vapour to outside the brewhouse. Disposal of the hop-back vapour in this way appreciably reduces the maintenance of the building steel work, *etc.* Perhaps an interesting feature is that the hop backs are fitted with revolving rakes for putting out the spent hops. A small dip of weak worts is put into the vessel, the rakes revolved to mix



PARK ROYAL BREWERY FROM THE AIR

thoroughly the content, and then the hop outlet is opened to a centrifugal "free flow" pump which pumps the spent hops over to the by-products department for draining and drying. The same pumping system is used for returning hops to copper for alternate boilings. The hopped wort from the hop backs is pumped up to the wort coolers which are large open vessels about 26 ft. $\times$ 24 ft. and 6 ft. deep, constructed in copper bearing mild steel and located on the top floor of the brew-house. They are open to the atmosphere. The wort lies here where atmospheric cooling is allowed to lower the temperature to 175° F., which ensures that the temperature, when running down, does not fall below a safe figure. The wort is discharged into the wort coolers over aeration hoods.

Regarding liquor storage in the brewhouse, there is a bank of three high receivers at the top of the house for holding liquor at 205° F. Each vessel has a capacity of 500 barrels. Another high receiver is isolated for taking the hot return refrigerator water from the wort refrigerators in the fermenting house. Below the high receivers there are five 500 barrel low receivers taking the condensate from the jet condensers and the weak wort return from the draining of the spent grains and hops.

The total liquor storage capacity of the brewhouse is about 5,000 barrels, *i.e.* about 1.6 times the average brew. For heating up, the liquor is circulated from the low receivers through tubular steam heaters and thence to the high receivers, although either high or low receivers can be circulated through the heaters if necessary. There are three tubular heaters each capable of heating up 400 barrels of water *per* hour from 130° F. to 210° F. when supplied with steam at 27 lb. p.s.i.g.

The inside walls of the brew house are finished in white glazed hollow bricks to give a good surface for cleaning and to provide reflected light and a degree of insulation.

The Fermenting House.—This had to be designed for continuous daily brewings of 3,000 barrels *per* day. After the appropriate stand in the wort coolers, the wort is run down by gravity through a 5-in. bore main at a rate of approximately 340/400 barrels *per* hour to centrifugal pumps which pass the wort through a battery of plate-type refrigerators on the third floor of the fermenting

house, where it is cooled to the required temperature. On its way to the fermenting tun, the wort is led to glass-lined troughs below the store, or pitching yeast presses, where the required weight of yeast is dropped into the flowing wort prior to its discharge into the fermenting tuns.

There are five 1,260 barrel tuns and six 630 barrel tuns, making a total of 11 in all. This capacity allows three brews to be accommodated simultaneously in the house and still have available one of each size for maintenance and Excise regauging. There are no attemperating coils in the fermenting tuns.

About 12 hours after the tun is full a certain quantity of gyle is drawn off to storage tanks and kept at a temperature of 40° F. by circulation through plate-type chillers cooled by brine at 25° F. When the desired fall in gravity has been attained, which is usually in about 65 hours, the tunnage is started and the beer is pumped up to the skimmers located on the three top floors of the fermenting house. Originally, the skimmers were fitted with attemperating coils for checking fermentation, but in recent years these have been removed as part of a "cleaning up" programme, and the skimmers now really serve the purpose of auxiliary fermenting tuns, the final cooling being carried out in a battery of plate-type refrigerators very similar to the wort refrigerators. The beer is in the skimmer about 24 hours, during which time up to six yeast crops are skimmed off at various intervals, the store or pitching yeast usually being taken from the earlier skimmings. Immediately after the final yeast skimmings the beer is pumped from the skimmers through the beer refrigerators and run down to the storage vats in the vathouse.

The wort refrigerators are of the multi-plate type of standard pattern, arranged in six lines on the third floor of the fermenting house, each line consisting of two sections of 36 plates, arranged in passes of nine plates. The plates have a cooling surface of 5.5 sq. ft. each. Each line of two sections has a capacity of 60 barrels *per* hour, making 360 barrels *per* hour in all, which allows the worts to be cooled in about 10 hours.

All pumps, both wort and water, are of the centrifugal moderate-speed type with variable speed D.C. motors for regulation and temperature control as may be necessary, the pumps being designed with a characteristic

curve ensuring that the discharge pressure does not exceed 35 lb. p.s.i.g. in order to avoid trouble with the plate joints.

To avoid undue scale on the plates, the wort refrigerators are taken down three times a year and the plates dipped in a 25 per cent. solution of caustic soda, after which they are thoroughly brushed and washed. The cooling water services consist of direct water with a winter temperature of 50° F. and a summer temperature of up to 70° F., and artificially chilled water at 45° F., the latter being used in summer. The seasonal temperature ranges are as under:—

SUMMER

			Direct water cooling.	Chilled water cooling.
Wort	..	° F.	175 to 78	78 to 55
Water	..	° F.	130 from 70	65 from 45
Water	Ratio	..	1.6	1.5
Beer				

WINTER

			Direct Water cooling.
Wort	..	° F.	175 to 55
Water	..	° F.	130 from 50
Water	Ratio	..	1.5
Beer			

From the wort refrigerators the wort runs down from the yeast troughs already referred to, to the fermenting tuns arranged on the floor below.

The tuns are of the totally-enclosed type and are constructed in Kauri pine, selected for its very well-known properties of hardness and smooth grain and low coefficient of heat conduction, the large ones being of 1,260 barrel capacity and are 28 ft. long × 26 ft. wide × 20 ft. deep. The small tuns are of 630 barrel capacity; they are 28 ft. long × 13 ft. wide × 20 ft. deep, the liquor dip in each case being 14 ft. 6 in. The ceiling of these vessels is formed by the reinforced concrete floor above, the underside of which is panelled in white glazed tiles.

No provision is made for the collection of CO₂ gas, but the gas flows over and through ports below the door sill to a gas trunking system connecting the fans which discharge the gas to atmosphere over the fermenting

house roof. For removal of gas after tunnage, portable reinforced rubber pipes of 6 in. diameter and long enough to reach the bottom of the tun are connected up to the gas trunking system with quick bayonet fastenings. A tun can be freed of gas within half an hour to enable men to enter for washing down. No detergents are used in washing.

It may be mentioned that difficulty has been experienced in recent years in these days of low gravities and their attendant troubles, in keeping the surface of the timber bacteriologically clean, and to overcome this it has been decided to line them with sheet metal. The first choice was stainless steel, but owing to the prolonged delivery dates and last, but not least, the very heavy cost involved, aluminium has been adopted. The lining will be of 6 gauge thick aluminium sheet 99.5 per cent. purity to B.S.S. A3 of welded construction with felt and bitumen backing, and the necessary inlet and outlet connections will all be "cleaned up" to remove, as far as possible, all corners where infection could possibly find a lodging.

Originally, the tuns were roused with mechanical power-driven paddles arranged on the floor of the tun, but it was almost impossible to keep these even reasonably clean and they are now being replaced by compressed-air rousers, which consist of a venturi tube in aluminium in the throat of which is arranged a ¾-in. nozzle. The potential energy of the compressed air is converted to kinetic energy in the venturi, thus inducing movement of the liquor mass. The unit is small and compact and readily removable for cleaning and is arranged on the floor of the tun. The air rouser gives excellent results, promoting vigorous circulation which can be easily controlled by the air valve, the operating pressure of the air being about 15 lb. p.s.i.g.

As the top fermentation process is used, means are provided for the mechanical removal of yeast from the beer surface. This is done in skimmer, of which there are 24 arranged on the three top floors of the fermenting house. The skimmers are large shallow cast-iron vessels each of 425 barrels holding capacity and are 57 ft. 8 in. long by 12 ft. wide and 4 ft. 3½ in. deep. When the gravity in the fermenting tun has reached the pre-determined figure above primary gravity, the beer is pumped to the overhead skimmers. Yeast is collected by the dropping system, the

skimmers being worked in vertical banks of three vessels.

Of the eight skimmers occupied by a brew, one is a balance vessel fitted with the orthodox parachute or movable yeast hopper. Ordinary skimmers have a yeast trough on the end of the vessel, and the yeast is manually skimmed to the trough and dropped into the yeast collecting vessels below each bank of three skimmers. Each skimmer yeast trough is fitted with two outlet chutes and a portable plug which allows the yeast from the skimmers to be passed down selected chutes to the yeast collecting vessels below. In this way a yeast crop for pitching can be kept isolated.

There are two stainless-steel enclosed cylindrical yeast collecting vessels each of 200 cu. ft. capacity, *i.e.* 400 cu. ft. for three skimmers holding some 1,275 barrels of beer. These collecting vessels are fitted with internal power-driven fob breakers for degassing the yeast by cutting it down as it enters the vessel. Continuous yeast collection can be carried out as one collecting vessel can be blown to the yeast presses while the other is being filled. The capacity of each pair of yeast collecting vessels is sufficient to take about 65 *per cent.* of the total yeast from three skimmers, as the yeast "broken down" by the fob breakers is about the same density as ordinary liquid yeast.

As there are eight banks of skimmers there are 16 yeast collecting vessels in all, arranged in two aisles one on each side of the house. The vessels are equipped for pressure evacuation by air at 45 *lb.* p.s.i.g., the yeast being blown to filter-cloth yeast presses. There are two blowing mains, one for "pitching," *i.e.* yeast used in the brewing process, and the other for surplus yeast. This ensures that a selected "pitching" crop can be isolated from collection in skimmer to pressing. The yeast-blowing mains are of tinned copper with rubber diaphragm valves and flexible rubber connections for connecting one vessel to either "pitching" or surplus yeast lines. The yeast vessels are scalded out with hot water at 190° F. after each brew.

The yeast presses are of the standard filter-cloth type cooled with chilled water at 45° F., there being one bank of four 9 *cwt.* presses for store or "pitching" yeast, this quantity being sufficient for two brews, which is necessary to maintain brewing over holiday week-ends. For the surplus yeast, there are seven 16 *cwt.* presses cooled with chilled water. All the

above weights are of pressed yeast. The barm beer from the press is collected in welded mild-steel enclosed collecting vessels. All the bottoms from the fermenting tuns and the fermenting house vessels are collected in stainless-steel enclosed cylindrical-bottomed vessels and blown to the surplus yeast presses.

The Vat House.—From the beer refrigerators in the fermenting house the beer is run down by gravity to the storage vats in the storage vat house, which is virtually a storage cellar, and to prevent any undue rise in temperature the building is lined with special insulation bricks of standard brick size. The flat concrete roof is asphalted and covered with white spar chippings in bitumen to reflect the heat of the sun. There are no windows and results have generally been most satisfactory, the temperature of the liquor in vat being practically unaffected in mid-summer. The vats are constructed of oak and hooped with copper bearing mild steel, there being 24 large vats, each with a capacity of 1,150 barrels, and eight smaller ones having a capacity of 870 barrels each.

The pipe lines, as in the fermenting house, are of tinned copper and there is nothing of particular interest in this building excepting the large positive displacement pumps which are used for transferring the beer from the storage vat house to the elevated racking vats. The choice of positive displacement pumps was determined by the variation in pumping head arising from the storage vats being 20 ft. deep and the racking vats some 18 ft. deep arranged above them, the transference from full to empty vats involving a difference in head beyond the characteristic curve of the standard centrifugal pump when maintaining the necessary output.

Cask Cleansing and Racking Shed.—The cask cleansing and racking shed is laid out and planned to take advantage of the natural fall of the ground from south to north with the result that casks will roll of their own volition from one machine to another throughout the length of the cleansing and racking bank.

There are five cleansing lines, each capable of handling 100 hogsheads *per* hour consisting of:—

- (a) An external washer, with clamping brushes, the casks being mechanically revolved.

- (b) Internal washer of the revolving nozzle type supplied with high-pressure water at 200 p.s.i.g. at 200° F.
- (c) A bank of 10 steaming nozzles fed with steam at 5 p.s.i.g. for three minutes *per* cask.

Common to the cleansing lines is a cooling floor where the steamed casks stand to "cool off" for 12 hours before passing forward to the rackers. There are four racking machines of standard gravity type, each capable of dealing with 140 hogshead *per* hour. For checking the capacity of casks coming from the hoop drivers there are two cask-measuring machines which check the net holding capacity by weight of water and which have a dial on which is indicated the variation from the standard weight. The daily routine is that the empty casks are received on the "high" end of the bank where defective hoops are dealt with on the hoop drivers and all casks pass through the line of washing machines in sequence.

The necessary hot water for internal cask cleaning nozzles is provided by two steam-heated water heaters, the hot water discharged from the internal-nozzle cleaning machines being collected and re-used on the outside cask washers, thus affecting a useful economy in heat.

The Cooperage Shops.—The cooperage shops are located adjacent to the cask cleansing shed, the machinery and equipment being of more or less standard type. The shops are laid out to cope with all repairs and new casks required for the Park Royal trade.

Pipe Lines and Valves.—A programme of simplification of piping circuits on the liquor side of the brewery mains system with a view to attaining more sterile conditions is being carried out; a few remarks on this may be of interest. Regarding the material for mains for carrying wort, beer and yeast, tinned copper has been found to be quite satisfactory over a long period, and the conclusion was reached that there were no economic or practical advantages to be obtained by changing over to stainless steel or aluminium at this stage, particularly as the elimination of a large quantity of copper main left ample stocks for realigning the various runs of mains. Stainless steel offered advantages from the point of view of smooth bores and reduced corrosion rate, but it is susceptible to the heat treatment necessary in welding and it is much

more difficult to work to shape than copper. More or less the same arguments apply to aluminium.

The question of joints is an important one in brewery beer circuits, and we are dispensing as far as is practically possible with the flange joint and rubber insertion ring, the latter being a serious source of infection trouble, as the rubber ring squeezes out into the bore of the main, forming pockets of infection. As far as possible, welded circumferential joints will be used and flanged joints will be used only at terminal points and valve connections. Welding methods to-day have reached very high standards by the adoption of the Argon-Arc, atomic-hydrogen and other methods, and these systems of welding in intelligent hands can produce good internal surfaces and also oxidation-free welds. It is necessary, of course, that the welder for this class of work is an intelligent man who has received some special training in the work.

For flanged joints it is proposed to adopt bolted gun-metal flanges brazed to the copper tube with serrated joint rings of phosphor-bronze, more or less in accordance with high-pressure steam joint practice. The advantage of the serrated metal ring joint is that it gives a metal-to-metal contact and maintains continuity of the bore and gets rid of the objectionable rubber ring.

It is proposed to sterilize all mains with low-pressure steam, which will subject the pipe lines to expansion and contraction stresses which will be met by expansion bends and flexible hangers again on the lines of steam pipe practice. When the mains are being hung they will be anchored at fixed points and closure spaces left equal to half the calculated expansion movement and the mains pulled up cold.

On the original installation pipe bores were fixed to give an average velocity of 4–5 ft. *per* sec. on a maximum flow, but practice shows that the mains were frequently running only half-bore and that as a result, a drying-out process was occurring, leaving a deposit on the walls of the pipe. To overcome this the velocity will be increased to 8–10 ft. *per* sec., which will promote turbulent flow and also, it is hoped, have a slight scouring effect which should prevent, or at least appreciably minimize, deposition. The mains will be hung to definite falls for drainage purposes which as far as possible will not be more than 1 in 40.

Like most breweries, we originally installed the expensive and heavy gun-metal plug cock on beer and yeast lines, *etc.*, but it has been found in practice to be a veritable bug-bear in all senses of the word. Furthermore, there was endless trouble in maintaining tight plugs when we commenced scalding mains owing to expansion and contraction. It has been decided to replace all plug cocks and gate valves throughout the brewery by the rubber diaphragm type of valve, which in addition to being much cheaper, presents no difficulty in maintaining tightness under intermittent high temperature conditions, and is easily maintained, the replacing of the diaphragms being almost a matter of minutes. On beer lines the diaphragms will be replaced after a fixed period of service which will be well within the useful life of the diaphragms. A modification of the standard rubber diaphragm valve has been designed which will obviate the pockets of liquor in the "dead ends" of pipe junctions and bifurcations, which are a frequent source of infection and loss of beer.

The problems of sterilizing mains can be resolved into (a) the positive removal of all deposits; (b) the destruction of all bacteria. The former can be carried out by the simple expedient of flushing out and the second by the application of heat at a "killing" temperature over a definite period of time, or by chemicals. It has been decided to adopt a combination of flushing out with cold water followed by low-pressure steam sterilizing, the latter being carried out on a definite temperature-time basis to ensure that the pipe and valve metal will be maintained at not less than 180° F. for 10 minutes.

The decision to adopt steaming in preference to scalding by hot water was taken for economy of heat and ease of application. When using hot water for sterilization purposes, it is necessary to maintain a flow to make up the heat loss occasioned by radiation and convection, and furthermore, the flushing must be vigorous enough, which means velocity and quantity, to remove deposits. This practice involves a very high consumption of hot water and is extremely wasteful of heat. With low-pressure steaming there is no need to have a flow of steam through the pipe owing to the large latent heat content of the steam, which means that the steam can give up heat without a drop in temperature. One charging of the main is usually sufficient

to maintain the required temperature over the required period.

Steam and Power.—The whole of the steam and electrical requirements are generated in a central power plant, the decision to adopt private generation being largely due to the process steam requirements of the brewery. As is well known, electric power generated on back-pressure turbo-generators is substantially cheaper than power generated on the condensing steam turbo-alternators of the large grid stations, owing to the loss of latent heat in the condensers to the river cooling water. The back-pressure power generation system has an average thermal efficiency ranging from 50 to 70 *per cent.*, while a similar figure for the large grid power stations is of the order of 27 *per cent.*

The power station plant was, therefore, designed to correlate the power and process steam demands, provision being made for the utilization of surplus back-pressure steam in a condensing turbo-generator. The station is designed to be completely independent of any external services beyond those of water and fuel. It is self-contained in its own steel-framed and brick-panelled building with boiler and turbine-houses and basements and annexes for the auxiliaries.

Coal can be delivered by rail, road or canal. It is discharged into the boot of a gravity bucket-conveyor which delivers it to the coal bunkers which have a capacity of 900 tons, or three weeks' supply. Oil fuel is delivered by road and stored in oil-fuel storage tanks with a holding capacity equivalent to four weeks' supply; the tank farm being remote from the boiler-house, the oil is pumped across to service tanks near the boiler-house. The normal maximum demand on the boiler-house is 70,000 *lb.* of steam *per* hour.

In the boiler-house are four water-tube boilers each with an economical rating of 30,000 *lb.* of steam *per* hour at 200 *lb.* p.s.i.g. and 500° F. All four of the boiler units were originally coal-fired, with mechanical stokers, but since the fuel crisis of the 1946-47 winter, two of the boilers have been converted to oil fuel firing. This means that total steam requirements can be met by either coal or oil.

Each boiler is fitted with its individual vertical tube economizer, forced-draught and induced-draught fans and cyclone dust collection equipment. There are three electrically-driven feed pumps, and one steam turbine-driven pump for emergency purposes. The

feed-water system incorporates combined de-aerators and feed heaters which deal with the water on its way to the economizer. The make-up of the feed water is about 25 *per cent.* of the total required as 75 *per cent.* of the condensate is returned from the brewery. Make-up is taken from the secondary circuits *ex-reservoir* and treated in a lime-and-soda water softening plant before it enters the feed system.

The main generating plant consists of four geared turbo-generators, three of which (2-500 k.w. and 1-250 k.w.) are back-pressure units which take steam direct from the boilers at 200 *lb.* p.s.i.g., and exhaust to the brewery process-steam mains at 30 *lb.* p.s.i.g. The back-pressure machines all have an overload capacity of 25 *per cent.* for two hours. The fourth turbo-generator (250 k.w.) acts as a balancer between the power and process-steam demands, taking surplus exhaust steam and expanding this down to 28 in. vacuum. The redress of the balance in the opposite direction is obtained by duplicated reducing valves which come into operation automatically if the process-steam pressure is reduced to 27 *lb.* p.s.i.g. Under these conditions the reducing valves admit reduced high-pressure steam to the process mains through de-superheaters.

The generating system is direct current 3-wire 425 volts between the outer conductors. About 5,000,000 k.w.h. *per annum* are generated by the power station and the normal load is about 750 k.w., necessitating the running of one 500 k.w. and one 250 k.w. back-pressure set, but this load is increased in summer to 800-900 k.w. for brief periods when the refrigerating plant is in service. In the turbine-house basement there is a 30 k.w. diesel house generating set, the primary duty of which is to provide sufficient power to maintain one boiler on the line ready to start up the turbo-generators as quickly as possible after the plant has been shut down for its annual overhaul.

From the feeder panels on the main switch-board the major distribution to the brewery proper is effected by two-core and three-core cables, the former being used for 420 volt power supplies only, and three-core cables for both 420 volt power and 210 volt lighting supplies. The cables are laid underground to the various building blocks of the brewery.

There are over 500 D.C. shunt-wound motors having an installed capacity of about

5,000 k.w. capacity. For speed variation shunt regulators are fitted. All motor starters are of the push-button automatic contactor type.

The Refrigerating Plant.—In common with all breweries the demand for cooling facilities at Park Royal is heavy, the maximum demand in summer being of the order of 5,000,000 B.T.U. *per hour*, occasioned principally by wort and beer cooling.

There are two sections of the refrigerating plant, one being the chilled water services at 45° F. for wort and beer cooling, and the other a brine service at 25° F. for yeast and gyle cooling. The machines are CO₂ compressors of the high-speed single acting vertical totally-enclosed type. The choice of a CO₂ system was made on the grounds that in the event of a leakage it would be less harmful than in an ammonia system, although it was appreciated that its higher vapour-pressure characteristic would require a considerably higher operating pressure—1,300 p.s.i.g., in fact; and also that it would be slightly less efficient from the thermo-dynamic point of view than ammonia.

The plant for the chilled water services comprises five machines, two of which are capable of eliminating 2,000,000 B.T.U. *per hour* each when cooling water from 65 to 45° F. with condensing water at 70° F. which is the summer temperature of the direct water. The remaining three machines are capable of eliminating 1,000,000 B.T.U. each under similar conditions. The condensers are of the submerged type, while the evaporators are of the enclosed type, both of steel casings. There is one condenser and three evaporators to each unit. The compressors are direct-coupled to variable-speed motors, those for the large machines being of 200 h.p. and the small machines 100 h.p.

The brine machines are similar to the chilled water plant excepting that there are three machines each of 250,000 B.T.U. *per hour* capacity, direct-coupled to 50 h.p. motors. Each machine, however, has one condenser and one evaporator. Three brine pumps are provided for the circulation of brine to various sections of the brewery.

For charging the installation with CO₂ gas, a charging station has been erected outside the main engine room which enables six flasks of CO₂ to be charged into the plant at the same time. The charging lines are so inter-connected throughout the plant that

gas can be drawn from another machine to assist in the charging of another if necessary, so that during overhaul work it is not necessary to lose any gas as it can easily be transferred to one of the other machines.

The By-products Department.—The plant in the by-products department was designed to dry all spent grains, hops and surplus yeast for disposal as by-products such as cattle food, *etc.* For removing the spent grains from the mash tun, a dip of 60 in. of liquor or scald is put into the mash tun and the rakes given a few turns to mix the mass thoroughly and then the outlet is opened to a "free flow" centrifugal grains pump, which delivers the mixture to a drainage vessel in the by-products. A follow-up with about 25 barrels of scald is then pumped through to clean the mains.

For the reception of grains there are four of these vessels each large enough to take the spent grains from three mash tuns. They are circular vessels in copper bearing mild steel 20 ft. in diameter and 16 ft. deep fitted with a gun-metal slotted false bottom plate similar to the mash tuns, which allows the liquor to be drawn off and returned to the brew-house system. The drainage stand is usually about 12 hours, after which the grain is put out to screw conveyors for transference to presses and dryers, the grains at this stage having a moisture content of about 80 *per cent.* The grains are put out by a revolving arm inside the vessel, this arm being fitted with "pitched" blades. The revolving arm is slowly turned through a power-driven gearbox, the arm at the same time being lowered slowly by an "inching" device to give the blades a cut. The pitching of the blades moves the cut of grain outward to the periphery of the vessel where it falls through a port in the side to a screw conveyor below. There are 12 grains presses on the grains side each consisting of a tapered propelling screw running in a tapered perforated drum, the liquor being squeezed out through the perforations which are 22 gauge. In these presses some 40 *per cent.* of the moisture is pressed out. The pressed grains are discharged to a screw conveyor for transference to a bank of five cylindrical steam-heater driers of more or less orthodox pattern, each drier being capable of producing 10 *cwt.* of dried grains *per hour.* The drying medium is exhaust back pressure steam at about 27 *lb. p.s.i.g.* On average

performance the driers operate on a ratio of 3 *lb* of steam to 1 *lb.* of dried grains.

On the spent hops side a similar arrangement exists excepting that there are two draining mash tuns, four presses and three hop driers of similar type to the grains driers, having an output of 3 *cwt.* of dried hops *per hour*, and the steam ratio here is 4 *lb.* of steam to 1 *lb.* of dried hops.

The pressed yeast in the fermenting house is liquefied in tubular steam-heated heat inter-changers (designed for turbulent flow), the liquefied yeast which remains in the liquid state when cold being pumped across to the by-products to a reception tank above the yeast driers. There are four yeast driers of the steam-heated drum type, the dried yeast being scraped off by a knife edge; the output of each drier is 1 *cwt.* *per hour.* Here the steam ratio is 7½ *lb.* of steam to 1 *lb.* of dried yeast.

The by-products plant can deal with the whole of the spent grains, hops and yeast from a full brew in two shifts. On the grains and hop sides there are centrifugal fans and cyclones for collecting all dried material; bagging off plant is also provided.

Fuel Economy.—As most of those concerned with the brewing industry will concede, economy in fuel did not receive much consideration in pre-war days for the very good reason that as an item of production cost, fuel did not represent a particularly important percentage. However, times have changed, with the result that apart from national economy considerations, fuel costs in the industry are an item not to be overlooked.

The original plans for Park Royal did not include for the installation of any flow meters throughout the brewery itself, and in the power station, apart from the switchboard ammeters, the only meters installed were on one of the four boilers which was fitted up with the usual feed-water meter, CO₂ recorder and coal meter, the idea being that this would be the test boiler to determine the most efficient method of operation; once this was established, it was assumed that if the other three boilers were operated in the same manner, they would be equally efficient.

From the early days of starting up to the beginning of fuel economy, there was a simple check of the weight of coal delivered to the power station against the weight of feed-water evaporated, the thermal efficiency

being calculated. The calorific value of the coal was arrived at by sending a representative sample to a Research Laboratory, coal samples having been drawn from each shift during the week, these being mixed and quartered, and a representative sample made up weekly. Prior to the war years, the average thermal efficiency over the year of the boiler-house was about 80 *per cent.*

This system was satisfactory up to a point and gave reasonable results as far as the boiler-house was concerned, but as a basis for fuel economy as conditions in 1942 demanded, results were quite inadequate as it was not possible to know what was happening throughout the brewery. In common with most industries, fuel economy measures in 1942 were started by making a detailed survey throughout all departments using heat in any form, to decide whether flow meters and recorders could be usefully installed. An interesting point of this survey was that it brought to light the rather startling fact that the coal/beer ratio was varying between 45 and 65 *lb.* of coal *per* barrel brewed, this consumption being for all brewery processes and services.

A technical Fuel Officer was appointed and flow meters were installed on all major users of steam and hot water, the meters being of the indicating, integrating and recording type, the majority being fitted with 1,000-hour charts. A system of weekly analysis was inaugurated, from which the consumption of all heat-using services, *i.e.* steam, electricity, gas, *etc.*, could be assessed for each heat-user unit and also for each department, and any undue consumption readily indicated either for a particular item of plant or a department. To investigate any unusually high rate of heat use, the 1,000-hour indicating charts were referred to, allowing deduction to be made as to where the excessive consumption occurred and when; compared with the process programme of the day, the reasons for and why excess occurs are usually ascertained.

The installation of the meters cost about

£3,500, and in the first year of use they were primarily responsible for a substantial reduction in the coal bill equivalent to a value of £8,000. Factors for converting the coal, steam, electricity and gas consumption to B.T.U.s have been calculated and these with the standard analysis sheet enable the weekly figures to be prepared by non-technical personnel.

This method of metering and analysis has reduced coal consumption from 45–65 *lb.* of coal *per* barrel brewed for all brewing processes and services to a steady average of 30–32. As a matter of fact the basis of comparison is on B.T.U. *per* barrel brewed, which is a more reliable figure, as it makes allowance for the variations in the calorific value of the coal which to-day may be anything from 10,000 to 13,000 B.T.U. *per lb.*

A typical week's fuel consumption analysis is as follows:—

Department	1,000 B.T.U. <i>per</i> barrel.	<i>Per</i> <i>cent.</i>
Malt store	1.62	0.53
Brewhouse	116.80	38.29
Fermenting house	9.10	2.99
Refrigerating plant	9.80	3.22
Vat houses	14.47	4.75
Cask cleansing shed }		
Cooperage	53.90	17.67
By-products		
Administrative offices	16.80	5.51
Reservoir pumping plant	1.81	0.59
General stores		
Engineers' shops	1.45	0.48
A.C./D.C. generating set		
Condensing turbine loss	34.64	11.35
Power station auxiliaries	18.86	6.18
Distribution and other losses	25.84	8.64
	305.09	100.00

In conclusion, the author wishes to express his thanks to the Directors of Messrs. Arthur Guinness, Son & Co., Ltd., for permission to publish this paper.

MEETING OF THE MIDLAND COUNTIES' SECTION HELD AT THE MIDLAND
HOTEL, BIRMINGHAM, ON THURSDAY, 17th FEBRUARY, 1949

MR. B. WOOD in the Chair.

The following paper was read and discussed:—

THE TECHNICAL EVOLUTION OF THE BELGIAN BREWERY
DURING AND AFTER THE WAR

By Professor J. DE CLERCK

In Belgium during the present century top fermentation beer has been steadily supplanted by lager and draught by bottled beer. During the recent war gravities fell to 1,009–1,019 and beetroot up to 50 *per cent.* was effectively employed when sugar and malt supplies ran low. Post-war conditions have brought high taxation, reduced consumption and intense competition.

In malting, the main development has been the careful restriction of respiration towards the end of the growing period, the Saladin system being still the vogue. Other devices for reducing malting loss are being investigated. The work of Govaert, which has led to possible economies in hops, is concerned with the artificial transformation of humulon into the more soluble and bitter *isohumulon*, thus avoiding loss through the formation of non-bitter resins. Pure culture top yeasts have often been found to acclimatize themselves to worts of a given gravity, even a very low one, and the occasional use of a stronger wort to nourish the yeast is of doubtful benefit.

Problems in conditioning and maturing of beer are discussed and a description is given of a new patent cask which can be filled without loss of gas or exposure to air.

It is intended to give a description of the technical evolution of the brewery in Belgium, but it will be useful to say, beforehand, a few words about the economic evolution of the Belgian brewery, which influenced greatly its technical development.

Before the first world war, there were many breweries in Belgium, more than 3,000. Beer consumption was very heavy, more than 200 litres *per* inhabitant *per* year (44 gallons). Almost exclusively top fermentation beer was brewed and sold in barrels. A few breweries started making lager beer; their products were more regular; they gained in the favour of the public and soon these breweries grew to become very large ones. In between the two world wars, two-thirds of the breweries disappeared and it was chiefly the bottom fermentation breweries that extended. The consumers lost their taste for the flat draught beer and the top fermentation brewers readjusted their methods by conditioning their ales; so doing they succeeded in competing successfully with the lager type. The flat draught beers disappeared completely.

Beer prices going up steadily because of continuous rise in taxation, the consumption *per capita* fell to 175 litres (about a barrel). The sale of bottled beer grew continuously.

During the last war beer became necessarily very weak as the shortage of malt was severe, gravities ranging from 2.4 to 4.7 *per cent.* extract (sp. gr. 1.009–1.019). The small quantities of malt brewers had at their disposal were not sufficient to provide these gravities and it was necessary to look for other materials. At first, sugar was used, but these supplies soon ran out and finally beetroot was the only material available. The beet was cut in strips, dried on a kiln and then ground, so as to get grits or flour. For those light beers, beetroot gave reasonable results. A simple extraction was sufficient and very often beers were brewed with 50 *per cent.* malt and 50 *per cent.* beetroot. Fermentations were good and the foam on the beer was firm and persistent. Even today, certain breweries still use beetroots, because this material gives a good head; perhaps through the pectin it contains. This pectin often

produced a persistent turbidity in the tanks, but the flavour of the beetroot was not noticeable, because the beers were so light that they did not, in fact, contain much of this material.

In order to give a pleasant flavour to these war beers, the brewers used chiefly sweetening products, colour and hops, which were still plentiful, but the latter could easily produce an astringent taste in the light beers. Vinegar was also used. In Belgium the sour beers, like *Lambic*, still meet with a certain favour and blendings were made of *Lambic* with ordinary beer. As there was no *Lambic* left during the war, beer-vinegar was manufactured to take its place. Notwithstanding all these efforts to keep one flavour, if nothing else, in the beer, the consumption dropped to one half of the pre-war figure.

After these innumerable difficulties with which the brewers had to cope, all their hopes lay in the return of peace; but it turned out to be a deception. When beer came back to its normal gravity in the beginning of 1946, consumption went up, but the beer was too expensive, because the State had naturally raised the taxes and cereals remained very dear. The total of brewing materials used dropped to 70 *per cent.* of the quantities of 1938. Since, in Belgium, the excise duties are calculated on the weight of materials used, they are the only correct basis to appreciate the beer consumption. The beers remained, however, slightly lighter than before the war, so that it must be said that the consumption fell by 25 *per cent.* The reasons for this setback are naturally much discussed among brewers. The opinion is often expressed that the habits of the population have changed. In fact, more coffee, fruits and chocolate are imported into Belgium than before the war. Nevertheless, the principal cause must be the price of the beer, which is too high; indeed, considering the purchasing value, in glasses of beer, of the workman's salary, it will be found that it lost exactly one-quarter since before the war.

This fall in consumption is the cause of a grave economic crisis in the Belgian brewing industry. The means of production being so important, and competition so strong, each brewer tries to keep his customers, selling the best possible quality at the lowest price. It is this crisis which stimulates the technical developments in the breweries; they are dominated by the necessity to manufacture at a lower cost without impairing the quality.

Some of the innovations designed to achieve this will now be examined.

The brewing methods of the Belgian breweries differ considerably from those used in Britain; the lager beers win more and more ground and breweries are characterized by the great number of different types of beer produced. Every brewery manufactures six or seven different kinds and this diversity is necessary in a country where so much beer is consumed. The innovations in the Belgian brewery will therefore, perhaps not apply to the British brewery, but the principles may come in useful in many cases. Here it may be mentioned that some of these new ideas are still at the experimental stage, but the tendency remains always the same: to produce better at a lower cost.

Brewing Materials.—Great consumer of brewing barley as she was, Belgium did not produce any before the war. She only grew six-rowed barley, the better varieties of which were used for top fermentation beers. During the war, two-rowed barleys were sown, which gave a better yield and in this way the Kenia variety was introduced into Belgian agriculture. Today the brewer finds part of his two-rowed barleys at home. This is not an innovation, far from it; Belgium has simply caught up with some other countries in this respect.

Malting.—Maltings have not undergone any alterations in Belgium. Malting is chiefly done in Saladin boxes. As the capacity of the existing plants is already too high, no new ones are being built. Every effort is made to reduce manufacturing losses without spoiling the quality, and it is chiefly in the respiration of the grain and in the malt culms that economies are sought. The Kropff system has been well known for many years; its principle is to reduce the air supply for the germinating malt at the end of the malting. The enzymes formed at the start keep on modifying the malt at this stage, but the consumption of carbohydrates and the formation of rootlets are impaired through the lack of air. To make this technique successful, it is necessary to control the respiration of the grain. As mentioned above, the enzymes are formed at the beginning of the malting process, when the respiration grows in importance. When the latter moderates, the enzymes have been formed and aeration can then be reduced. In the

larger maltings respiration is controlled, and thus, without any special machinery, losses are reduced by 1 *per cent*.

Another method giving an even higher economy has been patented by P. Fröschel (*C.R. Congrès Internat. Ind. Ferm.*, Ghent 1947, p. 195). This author, a botanist, has studied the inhibiting substances present in barley. All species of grains contain soluble principles in their hulls, and these exert an inhibiting or a retarding influence on germination. These principles pass into the steep water, from which they can be extracted. On sprinkling the malt on the floors with these substances, respiration is reduced and with it the growth of rootlets. This procedure diminishes the malting losses by 2 per cent. It must be said that this method has only been tried out on an industrial scale, but has not gone beyond the experimental stage.

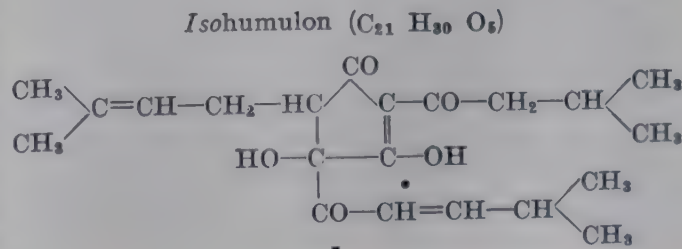
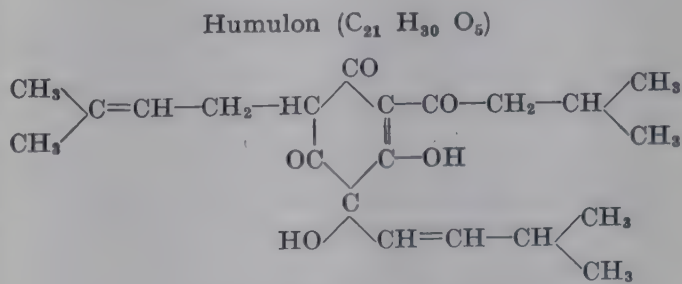
The malt kilns existing in Belgium before the war were sufficient to cover all needs and therefore, no new ones have been built. All are equipped with fans so as to allow deep layers and reduce the building costs. Before the war there was a tendency to work in very deep layers (1 metre), with powerful aeration and no turning over so as to complete the process in 24 hr. A hygrometer suspended above the layer indicates when the malt is dry. These kilns are much cheaper to build and require much less labour to run them. Quite good pale malts are produced on such kilns, but it is very difficult to get good dark malts in this way.

Brewing.—Brewing has hardly changed, but more and more attention is paid to liquor treatment. Certain breweries demineralize their water by means of ion exchangers. This constitutes an important factor in the improvement of the quality of the beer and counter-balances the effect of other economies which might have a less favourable effect.

Hopping.—If brewing has not been altered to any extent, the question of the hopping has attracted considerable attention. Study was made before the war of the practical use of the analysis of hops. In Great Britain the analytical valuation of hops is generally the determination of antiseptic power, according to the formula $\alpha + \beta/3$. This antiseptic power is of less significance for Belgian beers and it is the bitter value which is most important. From his experiments on the bittering power of the hop resins, Wöllmer

concluded that the bitter value could find its expression in the formula $\alpha + \beta/g$. Thorough tests, industrially, on the application of this formula have established with certainty that it has a great practical value. Calculating the hop rate according to its bitter value, much more regularity can be obtained in the bitterness of the beer and many Belgian brewers now calculate their hopping according to the bitter value given by the analysis. Buying hops, other qualities being equal, they choose those which have the strongest bitter value. It is obvious that the same bitterness is not produced in all beers with the same bitter value in the hops, because a number of factors may influence the bitterness and amongst these, chiefly the composition of the liquor. But once the right bitterness is fixed, the bitter value of the different hops is established and when another hop is used it is not exactly the same weight that is taken, but a proportion giving the same bitter value.

Another great novelty took shape in Belgium with reference to the hop extraction. Govaert (M. Verzele and F. Govaert, *ibid.* p. 292; *cf.* this Journ., 1949, 175) and his co-workers made a careful study on the chromatographic separation of the hop resins. This method made it possible, not only to measure more exactly the hop resins, but also to separate those present in the beer. From his investigations Govaert concludes that the bitterness of the beer is given by an isomeric transformation product of the humulon, which he calls *isohumulon*. The other transformation products of the resins would be without any value. The following are the formulae for humulon and *isohumulon*:



Isohumulon is much more soluble than humulon itself and is therefore the essential factor in the bitterness of beer.

When hops are boiled in the copper, only part of the humulon changes into *isohumulon*, the remainder becoming resins without any value. Govaert has thus elaborated a procedure, which is patented, and which has for its object the transformation of the humulon into *isohumulon* in the hop itself before it is added in the copper. This is done by means of treatment in an alkaline medium. In this way there are no more losses of humulon and the author claims a saving of 50 *per cent.* in the hopping. This method, which is quite new, has already been tried in several Belgian breweries. The hop saving is certain, but does not reach the 50 *per cent.* claimed. The experiments have not yet been taken far enough to know all the effects this procedure may exert on the other qualities of beer.

Cooling.—Here the Belgian breweries have turned their eyes to Britain. The new refrigerators are all plate-coolers, which British breweries were first to adopt. In comparison with the Baudelot (vertical) refrigerators they allow a great economy in space and in labour and give a much greater bacteriological security. Up to now, only a few experiments have been conducted on the clarification of wort by means of centrifugal treatment or by filtration through diatomaceous earth (*kieselguhr*), such as is done in America. Centrifugal treatment seems able to replace the settling of the wort on the surface cooler, and avoids considerable spoilage. However, existing plants will not soon be worn out, and therefore a long time will pass before centrifuges will take their place. Filtration through *kieselguhr* has been tried, principally to accelerate the maturing of the beer in the lager cellars. Further reference to this subject will be made below.

Fermentation.—Unlike British breweries, nearly all Belgian breweries now work with pure yeast cultures, both in top and in bottom fermentation. This certainly gives more regularity in the brewings and makes possible the choice and maintenance of those yeasts which are most favourable in each brewery. Evidently, when starting to ferment with pure yeast, the best results are not obtained right away. Several strains must be tried before finding a good one and it often takes quite a long time. The composition of the

brewing materials may sometimes necessitate the choice of another yeast, because the attenuation goes too far or not far enough. But when there is available a series of pure yeasts of good quality as regards the flavour of the beer, the attenuation, *etc.*, it is always possible to propagate the one which suits best, and this is a great asset. The strain of yeast does not mean everything; the acclimatization also plays a definite part. In this respect some astonishing happenings were witnessed during the war.

In the first period of the war, Belgian breweries received important quotas of sugar as brewing material. Certain breweries, which were running out of malt, added more and more sugar, fearing all the time that the fermentations would get weaker; but nothing abnormal happened. At one brewery the amount of sugar reached nearly 70 *per cent.* of the materials, for a wort of 5.4 *per cent.* extract (sp. gr. 1.021), and the yeast kept on fermenting nearly normally. It may be mentioned that it was a pure ale yeast, isolated from an English yeast. Suddenly the sugar allocations stopped and it was necessary to come back to malt alone. Right away, the yeast did not rise to the surface any more and remained in suspension in the beer, the attenuation remaining normal. It took some time before it again became acclimatized to the malt and behaved normally.

Another acclimatization phenomenon: The gravity of the beer was strictly limited to 4.7 *per cent.* extract maximum, but with the idea that yeast could not maintain its vitality in such weak worts the brewers were allowed to ferment heavier worts, the beer having to be diluted afterwards. For lager beer, it was generally necessary to adopt this procedure, but for the top fermentation it was realized that the fermentations were satisfactory notwithstanding the weak wort, and it was sufficient to regenerate the yeast from time to time in a stronger brew. After a certain period it was noticed, however, that this regeneration did more harm than good to the fermentation and that it was preferable to let the yeast get acclimatized to the low gravity. In this respect a certain yeast may be mentioned, that since the war has been fermenting exclusively worts of a gravity never superior to 2.6 *per cent.* (sp. gr. 1.010) extract without renewal, and the fermentation is still perfect. It seems therefore preferable for a yeast to ferment always worts of the same gravity.

A third acclimatization phenomenon has been noticed during the war, but here it concerns bacteria. At first, when the gravity of the beers dropped suddenly, rods and sarcina were no longer found, and the phenomenon got such that it was necessary to fetch old cultures of these organisms to show to students. When the war had lasted two years, these micro-organisms appeared again and progressively their number grew till the end of the war. As soon as the beers were brought back to their normal strength, they immediately became more virulent.

Storage (Lagering).—The war beers, being very weak, were much more sensitive to oxidation because of their deficiency in reductones. The flavour of these beers was easily affected by oxidation. For this reason, the brewers soon learned, especially during that period, how to preserve their beer from the influence of the air and to minimize oxidation. Reductones were manufactured with sugar and added to the beer. By so doing it was possible to avoid the astringent taste, which results from the oxidation of the tannin. When a sufficient amount of reductones is added to beer, the oxygen is fixed before it can oxidize the tannin, and the flavour of the beer remains purer.

Certain Belgian brewers find themselves under the necessity of shortening the storage of their beer; some because they keep on growing in importance notwithstanding the general crisis, others because in the pre-war days they had specialized in conditioned top fermentation beers, which required a short period of storage only, whilst now they are asked to brew lager beer which normally has to be stored for a longer period. That is why studies are now being made of certain methods to shorten the lagering. Some of these are based on a slight oxidation of the beer after fermentation, with the object of destroying the "young" flavours and of precipitating the turbidity. This oxidation is followed by a carbonic acid saturation. Filtration of the wort through kieselguhr is another means to hasten ripening, adopted in the United States. This method certainly accelerates the maturation. One objection to this procedure is that it makes the beer thin, but experiments show that the beer becomes bright and tastes mature sooner, and does not become thin unless it is left for too long a period in the lager tank. In any case, there is always the

choice of filtering only part of the wort through kieselguhr.

Up to now, this procedure has not been sufficiently studied in Belgium and it is not certain yet that it will give beers as good as those matured normally. It is possible that the beers with a short maturation period require another yeast or a different kind of fermentation.

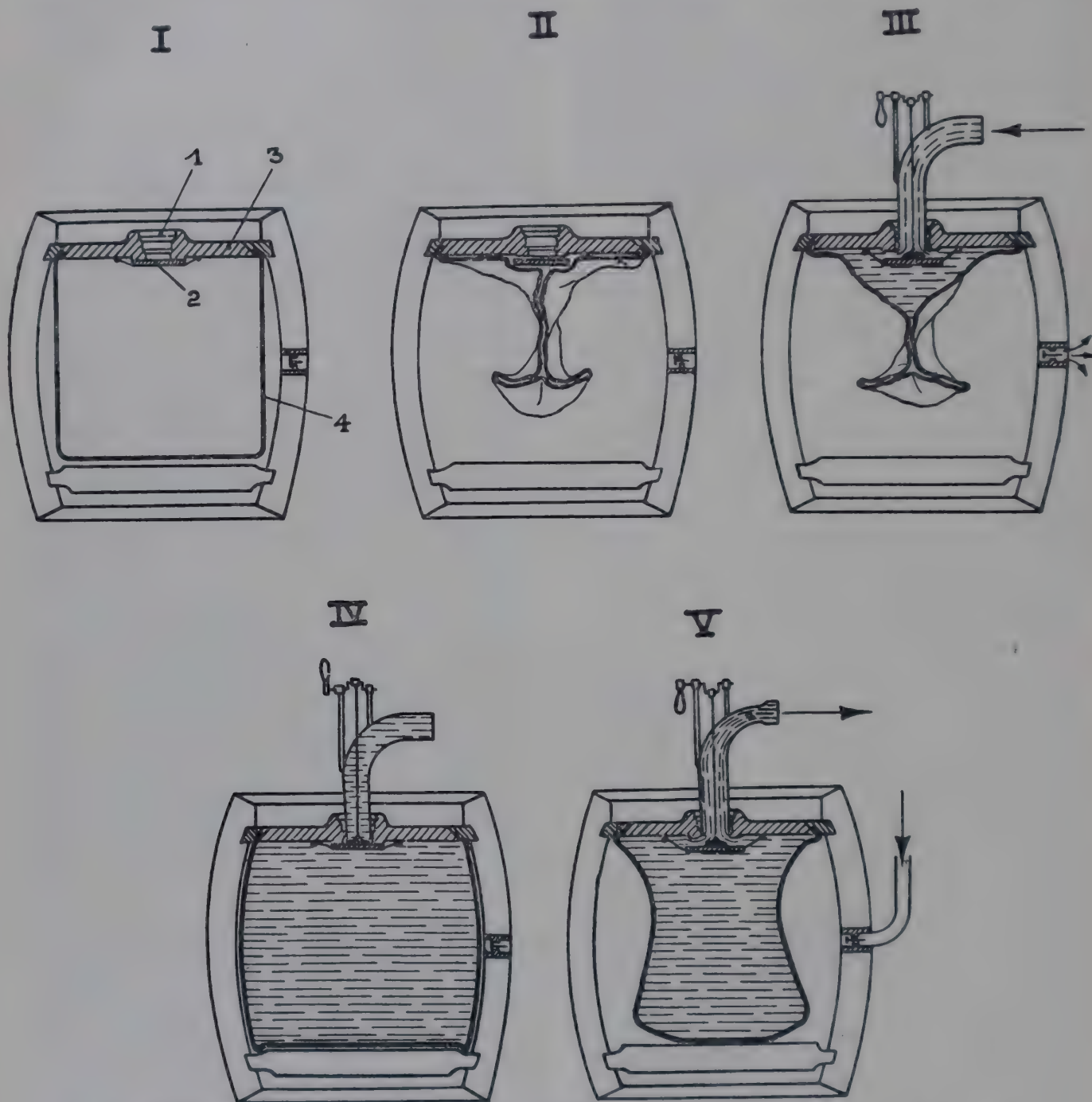
Filtration.—Since the war, beer filtration has been the object of many experiments. The pulp filter, which was generally used, demands too much labour, produces too much beer loss and is a cause of numerous infections. To take its place, there is choice of the plate-filter, the kieselguhr filter, and the centrifugal separator. All three offer the advantage of requiring less labour and producing less spoilage. However, the plate-filter gets easily clogged up, the kieselguhr filter lets yeast through, and the centrifuge gives less brilliant beer. Individual choice must be made in every special case. The kieselguhr filter is perfect for very turbid beers, but has to be followed by a plate-filter to avoid yeast-turbidity. The plate-filter is too quickly clogged when the beer is not sufficiently settled down. The centrifuge followed by a plate-filter is the easiest to handle and gives the least spoilage. This apparatus also offers the advantage that very clear beers or dark beers can be clarified satisfactorily by means of only a separator; but the output of a centrifuge is rather small. Generally, the choice today would be a kieselguhr-filter with a plate-filter for large outputs, and a centrifuge followed by a plate-filter for small outputs.

Racking.—As was said at the start of this paper, bottled beer is on the increase in Belgium. The bottling machinery does not differ from that in use in other countries. A start has been made in installing pasteurizers. Previously, beer was sold quickly, but now the turnover is slower; at the same time the customer has become more particular and it is necessary to aim at a longer stability. The pasteurizers have only been ordered and it is not yet known how the public will accept pasteurized beers. Anyway, the breweries are studying carefully the means to preserve their beers from the flavours and turbidities that may accompany pasteurization, and this before they adopt this innovation in practice.

Barrels also constitute an important

problem. The lack of oak wood and the high cost of wooden barrels were the cause of many experiments with metallic kegs. Trial has been made of coated steel, aluminium alloys,

shows, it is a barrel in which one of the ends has been replaced by a metallic plate (3) in which is inserted, around a big oval flange, easily removable, a rubber pouch (4) of the



(1) Empty barrel not under pressure: 1—bung, 2— valve, 3—metallic plate, 4—pouch.
 (2) Empty barrel under pressure. (3) Barrel filling. (4) Barrel full. (5) Barrel emptying.

and stainless steel, but the perfect metallic barrel has not yet been discovered.

A novelty in the way of barrels has been patented in Belgium during the war. It is a barrel with a pouch, but its use has not left the experimental stage so far. As the drawing

same capacity as the barrel. In the centre of this metallic piece is located the filling bung (1), which is opened by lowering the valve (2). To fill the barrel the pouch is first flattened against the plate, blowing air under pressure in between the sides of the barrel

and the pouch. The beer pipe is then fixed to the bung and, letting the pressure escape from the barrel, the beer runs into the pouch without loss of carbonic acid, without foam, and without coming in contact with any air. In order to empty the barrel, a beer faucet is attached and the pressure is again admitted around the pouch. The wood of the barrel is made impervious by means of a rubber coating so as not to lose any pressure. It would also be possible to draw the beer off by using water pressure around the pouch.

The advantages of this system are numerous. There is no need to use an *isobarometric* filler; no pitching of the barrels is required; the staves can be made of beech instead of oak; the beer is easily cooled by putting ice on the metallic face; the beer does not loose its gas, when the barrel is not hermetically sealed; it does not get infected through the organisms which thrive in between the pieces of wood and which it is impossible to eliminate; the beer does not get spoiled even if it takes several weeks to empty the barrel. The great difficulty in this apparatus has been to find a

kind of rubber perfectly neutral to the taste; it now seems that this difficulty has been overcome.

This barrel is still in the experimental stage; it would appear that the results are such as to indicate that this barrel would be capable of stemming the very expensive sale of bottled beer.

These, then, are some of the different technical problems which the Belgian brewery has to solve. They are at the origin of numerous theoretical problems still to be studied. The heads of the Belgian brewing industry have understood the unavoidable necessity of scientific and technical research to keep their industry in pace with progress. With this object, they have just established an important research centre, subsidized by the whole of the brewing and malting industries, thus following the example of the Institute of Brewing.

Université de Louvain,
Belgium.

COMMUNICATIONS

JOINT ACTION OF α - AND β -AMYLASES. II. INFLUENCE OF $\beta : \alpha$ RATIO AND OF TEMPERATURE ON REDUCING GROUP PRODUCTION FROM STARCH

By I. A. PREECE, M.Sc., Ph.D., F.R.I.C. and M. SHADAKSHARASWAMY, M.Sc., Ph.D., A.R.I.C.

It having previously been shown that the proportion of $\beta : \alpha$ amylase in brewery malts may vary over a range of 2.5 : 1 to 6 : 1, it has now been attempted to investigate the influence of such variation on starch hydrolysis as measured by reducing group production at infusion mashing temperature.

It has been shown that in conversions in which the proportions of amylase to starch are equivalent to those in malts of D.P. 15° Lintner and above, the $\beta : \alpha$ ratio is of little consequence for reducing group production so long as it exceeds $\sim 3\frac{3}{4} : 1$; for lower D.P. equivalence, however, a lower ratio of $\beta : \alpha$ may give a higher production of reducing groups than a higher ratio. Temperature sensitivity in the range 63-67° C. (145-153° F.) increases with either fall in total enzyme concentration or in $\beta : \alpha$ ratio. Thus, to increase the temperature in this range is equivalent in respect of reducing group production to using either a lower D.P. or a lower $\beta : \alpha$ ratio. The need for care in attempted direct application of these results to practical mashing conditions is emphasized.

INTRODUCTION

In the first communication of this series (this *Journ.*, 1949, 103), the application has been reported of copper and ferricyanide reduction methods to the study of amylolytic activity with respect to both α - and β -amylases in malt extracts and in precipitated enzyme preparations. Thus, it has been confirmed that, in presence of α -amylase, ferricyanide gives high results as compared with copper methods, but correction is possible within reasonable limits of accuracy. Further, it has been shown (Preece and Shadaksharaswamy, *Biochem. J.*, 1949, 44, 270) that, under the conditions of diastatic power determination, reducing group production by the two components of "malt diastase" is additive; hence if α -amylase activity is determined, e.g., by the method of Preece (this *Journ.*, 1947, 154; 1948, 141), β -amylase activity can be found by difference from the D.P. It has accordingly been established from analysis of representative Scottish brewery malts that the $\beta : \alpha$ ratio in finished barley malt approximates to 4 : 1, though there is some spread on either side of this figure (2.5 : 1-6 : 1). Also, a consideration of the absolute and relative

values of α - and β -activity can give useful information as to the treatment of the malt during its preparation, especially on the kiln (Preece, this *Journ.*, 1948, 141; *Chem. & Ind.*, 1948, 611).

To assess more fully, however, the value of the D.P. and α -amylase activity determinations, it would be necessary to have information as to the starch-hydrolysing potentialities of mixtures of the two enzymes in the range of proportions likely to be met with in brewery malts, and also the behaviour with different ratios at different levels of total enzyme concentration and at different temperatures approximating to those used in typical infusion mashes in this country. It is the purpose of the present report to initiate a discussion of starch conversion under the conditions outlined and, in the first instance, with special reference to the liberation of reducing groups from starch.

The difficulties in the way of a complete investigation of this problem are considerable and have been briefly outlined elsewhere (Preece, *Chem. & Ind.*, 1948, 611). In mash-tun conversions the starch concentration is ~ 20 per cent., an inconvenient level at which to work in preliminary experiments

where it is desired to maintain the starch: enzyme ratio at a value comparable with that of mashing practice. In the present work, 1 *per cent.* soluble starch has been used as substrate and later extension of the work is desirable to ascertain the influences of starch concentration and of the nature of the starch on the results obtained. Furthermore, it cannot be too strongly emphasized that amylase action in the mash must be profoundly influenced by accompanying substances, including inorganic ions and the products of other concurrent enzyme actions. An investigation of the effects of concomitants must likewise be postponed until the principles of action under relatively simple conditions are themselves more thoroughly understood. It should be made clear, therefore, that this report relates to a preliminary study, but the methods followed and the results obtained should in due course be susceptible of extension to include such other operative factors.

EXPERIMENTAL

Enzyme Preparations.—Dry, precipitated preparations of α - and β -amylases were obtained from malt and barley, respectively, by methods previously described (Preece and Shadaksharaswamy, *Biochem. J.*, 1949, **44**, 270). β -amylase activity could not be detected in the α -amylase preparations. With the β -preparations, the ratio of β : α saccharifying activity (ferricyanide method) was in the least favourable cases $\sim 750:1$, whereas in other preparations no α -amylase activity could be detected; contamination with α -amylase even to the maximum extent indicated may be regarded as insignificant in experiments dealing with the joint action of the two enzymes. Further, incubation of either preparation with 1 *per cent.* maltose solution at p_H 4.6, 21°C. (70°F.), and for three hours, produced no measurable increase in reducing groups; hence the absence of maltase may be assumed.

Amylase Ratios and Concentrations.—In a previous paper (Preece and Shadaksharaswamy, *Biochem. J.*, 1949, **44**, 270), a unit of amylase was arbitrarily selected as the weight of preparation required to produce reducing groups equivalent to 12.5 mgrm. of maltose in 15 min. from 1 gm. of soluble starch in a total volume of 100 ml. at p_H 4.6 and 21°C (70°F.). This unit has been retained

in the present work, but it should be noted that, in the case of α -amylase, apparent maltose production as measured by copper reduction will be 10.0 mgrm. only, since the original standardization was carried out with ferricyanide which gives high results with this enzyme; with β -amylase, on the other hand, 12.5 mgrm. is correct by either method (this *Journ.*, 1949, 103).

It is of interest to know the Lintner equivalents of the amylase concentrations used for conversions. Considering the case of an α -amylase unit, production of a maltose equivalent of 10.0 mgrm. in 15 min., *i.e.*, 20.0 mgrm. in 30 min., would correspond to the activity of 2 ml. of a 5 *per cent.* aqueous malt extract (as in D.P. determination) prepared from a malt of 20.0/3.85° L. (*loc. cit.*). Assuming the average starch content of malt to be 57 *per cent.* (*cf.* Preece, *J. Incorp. Brewers' Guild*, 1941, **27**, 19), this amount of amylase would accompany 0.057 gm. of starch in the malt. A malt in which it accompanied 1 gm. of starch (as it does in the present conversions) would accordingly have a D.P. of $20.0 \times 0.57/3.85$, or 0.30° L. This then is the Lintner equivalent of 1 α -unit when acting on 1 gm. of starch. Similarly, the equivalent of 1 β -unit is 1.25×0.30 , or 0.375° L. Conversions were carried out with $x\alpha$, $x(2\beta + \alpha)$, $x(3\beta + \alpha)$, $x(4\beta + \alpha)$, and $x(5\beta + \alpha)$, where α and β signify units of the two enzymes as above defined, and the values of x used were 1, 5, 10, and 20. The unit (*i.e.*, ferricyanide) ratios, their equivalents as copper-reduction ratios, and the corresponding Lintner values of the mixtures are summarized in Table I.

Starch Conversion.—The standard temperature employed was 65°C. (149°F.), but conversions were also carried out at 63°C. (145°F.) and 67°C. (153°F.). The p_H was uniformly 5.4, which is not far remote from the p_H of a typical malt mash and is stated by R. H. Hopkins (this *Journ.*, 1925, 399) to be probably generally optimal for mashing from the point of view of beer production. The substrate for each conversion was 90 ml. of 1.11 *per cent.* (dry weight) soluble starch solution at a temperature somewhat above that desired, so that on adding the required mixture of enzymes contained in 10 ml. of water at room temperature, the desired conversion temperature was attained in the mixture in a few seconds. Conversion

TABLE I

β : α RATIOS INVESTIGATED AND THE LINTNER EQUIVALENTS OF THE MIXTURES AT THE TOTAL CONCENTRATIONS USED

β : α ratio { ferricyanide .. copper		0:1 0:1	2:1 2½:1	3:1 3¾:1	4:1 5:1	5:1 6¼:1
		Lintner equivalents (° L.):				
Relative total concentration of enzymes:	X1	0.3	1.1	1.4	1.8	2.2
	X5	1.5	5.3	7.1	9.0	10.9
	X10	3.0	10.5	14.3	18.0	21.8
	X20	6.0	21.0	28.5	36.0	43.5

TABLE II

INFLUENCE OF β : α -AMYLASE RATIO ON REDUCING GROUP PRODUCTION FROM 1 per cent. SOLUBLE STARCH SOLUTION AT p_H 5.4 AND 65° C. (149° F.)

(Reducing groups determined by ferricyanide method, and results expressed as apparent maltose per cent. of starch)

Relative total concn. of enzymes.	Conversion time (min.).	β : α ratio (copper values).				
		0:1	2½:1	3¾:1	5:1	6¼:1
X1	15	7.3	8.0	9.6	10.5	11.2
	30	10.0	10.9	13.3	14.2	14.9
	45	11.0	12.6	15.1	16.2	16.7
	60	12.0	13.5	16.4	17.3	18.2
	120	13.1	15.0	18.4	19.2	19.9
X5	15	26.3	36.3	39.1	47.5	51.8
	30	32.3	42.3	45.3	53.0	56.6
	45	33.7	44.6	46.9	54.2	57.5
	60	34.8	46.9	48.2	55.1	58.3
	120	35.5	47.7	49.3	56.6	58.6
X10	15	38.1	62.1	64.6	67.4	70.0
	30	41.7	64.8	67.4	69.1	71.4
	45	42.8	65.7	68.3	69.6	71.8
	60	43.5	66.7	69.1	70.0	71.8
	120	44.6	67.6	70.0	70.9	72.7
X20	15	42.4	64.8	71.4	74.1	75.0
	30	45.1	67.6	72.3	74.5	75.5
	45	46.6	69.0	73.2	75.0	75.9
	60	47.3	69.8	73.7	75.5	75.9
	120	50.0	70.3	74.1	75.5	76.4

was continued to a maximum of 2 hours in a thermostatically controlled water-bath.

Reducing Group Determination.—Aliquots of the conversions were pipetted at appropriate intervals into sufficient aqueous sodium hydroxide solution to give a total volume of 10 ml. at a p_H of 10.5–11, thus stopping the reaction. Reducing group determination then followed by the ferricyanide method

already described (Preece and Shadaksharaswamy, *Biochem. J.*, 1949, **44**, 270).

Results.—In Table II are shown the results at a constant temperature of 65° C. (149° F.) when the ratios of β : α are varied within the limits summarized in Table I and when the total concentrations of enzymes are also changed. It may be noted that a ratio of 3¾:1 and a total enzyme concentration of

TABLE III
INFLUENCE OF β : α -AMYLASE RATIO AND OF TEMPERATURE ON REDUCING GROUP
PRODUCTION FROM 1 per cent. SOLUBLE STARCH SOLUTION; p_H 5.4
(Reducing groups determined by ferricyanide method, and results expressed as apparent
maltose per cent. of starch)

Temperature (° C.).	Relative total concn. of enzymes.	Conversion time (min.).	β : α ratio (copper values).				
			0: 1	2½: 1	3¾: 1	5: 1	6¼: 1
63	X10	15	39.3	65.7	67.4	71.8	74.7
		30	42.8	69.2	70.5	73.5	75.4
		45	43.7	71.3	71.8	74.5	76.3
		60	45.0	72.2	72.7	75.4	76.7
		120	46.5	72.2	73.5	76.3	77.2
	X20	15	42.8	66.7	70.9	74.5	76.3
		30	45.1	70.3	72.8	77.2	78.1
		45	47.3	73.1	73.2	78.1	78.6
		60	48.2	74.0	74.1	78.6	79.0
		120	50.9	74.9	75.0	79.5	79.5
67	X10	15	37.0	41.8	48.2	52.6	57.5
		30	40.4	45.5	51.8	55.8	59.7
		45	41.0	46.4	52.2	56.2	59.7
		60	42.2	46.9	53.1	57.0	60.1
		120	42.6	47.8	54.0	57.5	60.6
	X20	15	41.7	60.2	61.6	65.6	70.9
		30	44.5	62.9	63.4	67.4	72.8
		45	44.9	63.2	64.3	67.8	73.7
		60	45.3	63.9	64.7	68.3	73.7
		120	46.6	63.9	65.6	69.6	75.0

X20 gives an enzyme-starch relationship closely comparable to that found in an "average" brewery malt (Preece, this *Journ.*, 1947, 154). The influence of varying α -amylase concentration when unaccompanied by β , and of varying the β when α is kept constant (X20) is shown in Figs. 3 and 1 respectively.

The influence of temperature change was studied by repeating conversions with total enzyme concentrations X10 and X20 at 63° C. (145.4° F.) and 67° C. (152.6° F.), with the results shown in Table III. Using results from Tables II and III, the influence of temperature for varying β : α ratios at X10 concentration for 1 hour conversions is shown in Fig. 4, whilst Fig. 5 shows the effect of total enzyme concentration for the constant ratio of 3¾: 1, also at 1 hour.

DISCUSSION

Perhaps the most striking feature of the results is the great initial speed of the reaction, this being especially marked at the

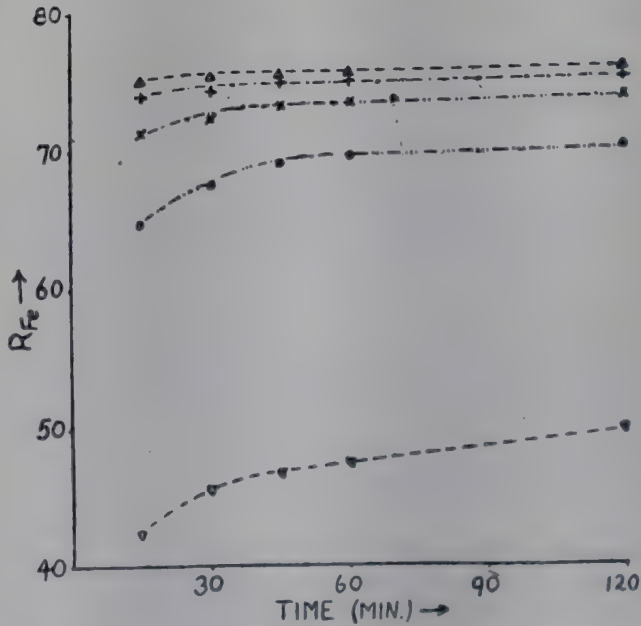


FIG. 1—Influence of β : α ratio on ferricyanide-reducing group production (R_{Fe}), when α concentration is constant (X20). Reading downwards, the ratios are (copper values) 6¼: 1, 5: 1, 3¾: 1, 2½: 1, α alone. 1 per cent. soluble starch, p_H 5.4, 65° C.

higher β : α ratios. There would appear to be no evidence that β -amylase is active after 15 min. at 65° C., subsequent slow action being due entirely to continued α -amylase activity, this proceeding to an extent determined by the proportion of susceptible linkages remaining; this proportion will be smallest—and hence the curves from 15 min. to 120 min. will be most nearly horizontal lines—with the highest proportions of β : α (Fig. 1) and with the highest total enzyme concentrations, such conditions favouring the nearest approach to the completion of rapid action in the first 15 min. Although β -amylase action is short-lived, it is significant that during its survival a relatively great liberation of reducing groups is possible, as evidenced by the positions of the joint action curves with respect to that for α -amylase alone (Fig. 1).

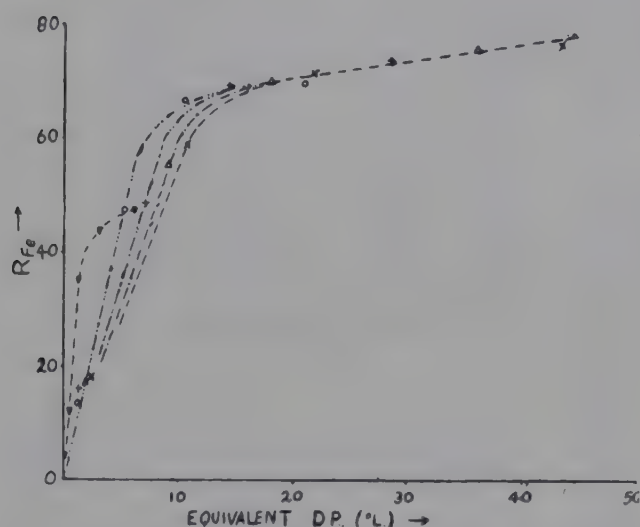


FIG. 2—Influence of equivalent D.P. on reducing group production (R_{Fe}). Reading from left to right the curves represent α alone, β : α 2½:1 (copper values), 3¼:1, 5:1, and 6¼:1. 1 hour conversions.

The influence of the β : α ratio on the extent of hydrolysis in a given time (1 hour) at 65° C. is shown in Fig. 2, where the results of Table II are presented in such a form as to show hydrolysis plotted against equivalent D.P. in the conversion mixtures at the different ratios investigated. In view of the great speed of the initial reaction and the small number of results available in that region, accurate representation of behaviour below a D.P. equivalent of 10 is not possible, but examination of the Figure and Table shows that, in general for a given D.P. and the range of ratios investigated, the pro-

duction of reducing groups increases in the region below D.P. 10–15 as the proportion of β -amylase falls; at the lowest values (below D.P. 5), α -amylase alone produces more reducing groups than even the 2½:1 ratio, though here the position is somewhat complicated by the exaggeration of reducing group production due to the use of ferricyanide determination, which will naturally be especially marked in presence of an overwhelming preponderance of α -amylase. However, even taking this into account, qualification required of the above statement is slight.

At the higher D.P. values, on the other hand, behaviour is different. Thus, for ratios of 3¼:1 and greater, and with D.P. values above 15, reducing group production is independent of the actual ratio, and increases steadily with increase in total activity, giving an increase of approximately 3 per cent. for each 10° L. rise; there is, however, evidence of a slight falling off as the R 80 stage is approached more nearly. With the ratio at 2½:1, reducing group production in the range above D.P. 15 appears to be somewhat inferior to that obtained with the higher ratios. Direct comparison is possible, despite the use of ferricyanide for the determinations, as the value of the ferricyanide/copper factor will show only minor change for the different mixtures at this range of conversion. It will be recalled that the brewery malts examined earlier (*vide supra*) had β : α ratios averaging 4:1, and it is to be expected therefore that the actual value of the ratio will have little influence on the production of reducing groups in the mash-tun. Nevertheless, it must not be overlooked that reducing groups may be associated with fermentable or unfermentable carbohydrate, so that constancy of reducing group production does not necessarily imply constancy of composition in the products formed. This aspect will be discussed in a later report.

Conversion results with α -amylase alone are of considerable theoretical interest. With the four enzyme concentrations used (65° C., p_H 5.4) the hydrolysis curves (Fig. 3) all show an initial rapid action, completed in 30–45 mins., followed by a slow, prolonged liberation of reducing groups; moreover, although the degree of hydrolysis attained in the rapid initial phase increases as the

enzyme concentration increases, the subsequent slow action proceeds at substantially the same rate in all cases. That the rapid dextrinization and the slow saccharification of a starch substrate might be expected to overlap to a degree varying with the conditions of experiment has been argued previously (Preece and Shadaksharaswamy, this *Journ.*, 1949, 103), and the present observations would appear to lend strong support to the virtual independence of these reactions. It is difficult to escape the conclusion that, of the two reactions, dextrinization is the more susceptible to temperature, so that with small amounts of the enzyme, inflection at 65° C. is reached far short of the generally accepted ~ 33 per cent. stage. Reason has already been given (Preece, this *Journ.*, 1948, 141) for the view that temperature alone up to 70° C. (158° F.) has little inactivating effect on α -amylase, and it has recently been shown (R. B. Alfin and M. L. Caldwell, *J. Amer. chem. Soc.*, 1948, 70, 2534; this *Journ.*, 1948, 341) that the slowing up of the action of pancreatic amylase is not due to dialysable products of reaction; the latter authors also note the dependence of the degree of hydrolysis reached on the amylase concentration, their conversions being carried out at 40° C. (104° F.). There would appear to be little if any published data on α -amylase conversions carried out at 65° C. under conditions approaching those used in the present work, but it may be suggested that differences in affinity of the enzyme for starch and dextrans (see, e.g., Hopkins, *Advances in Enzymology*, 1946, 6, 389; Alfin and Caldwell, *loc. cit.*) are hardly sufficient to explain the whole of the observed facts. It would seem unwise at this stage to exclude the possibility of "α-amylase" being in fact two enzymes or, if a single enzyme, having active groups of different function.

On this basis, it might well be that the α -amylase inhibitor envisaged earlier (Preece, *loc. cit.*) is provided by dextrans of intermediate complexity, these forming initially reversible associations with the dextrinizing groups of the enzyme, the association however becoming irreversible as time at a given high temperature, or the level of the temperature, is increased. Thus, with low concentrations of enzyme, such dextrans produced during the period of dextrinizing activity would be only slowly degraded

further. With sufficiently higher enzyme concentration the more profound disintegration resulting might suffice to complete this stage of the reaction before the rapid function was lost. That subsequent slow action takes place at substantially equal rates for all enzyme concentrations may be fortuitous; thus, if saccharification is progressively impeded as molecular complexity diminishes, the observed result is the one which would be expected, as the higher enzyme concentrations would have the least favourable substrate on which to act. If

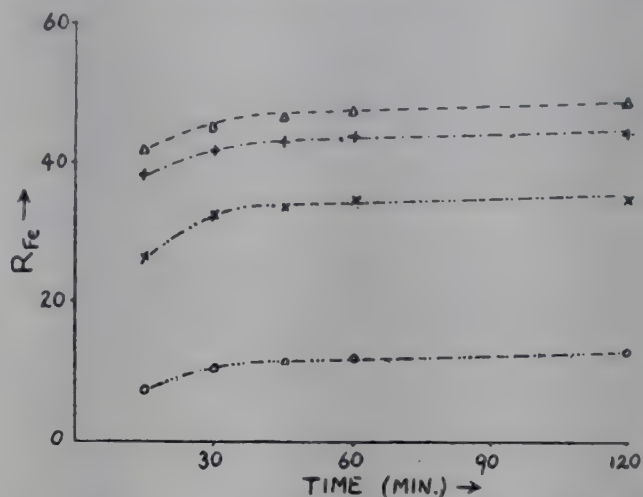


FIG. 3—Influence of varying α -amylase concentration on ferricyanide-reducing group production (R_{Fe} , apparent maltose per cent. of starch). The relative concentrations are, reading downwards, X20, X10, X5, and unity. 1 per cent. soluble starch, p_H 5.4, 65° C.

this hypothesis is correct, it might be supposed that the presence of β -amylase would enhance the stability of α by reducing the complexity of potentially inhibiting dextrans; evidence of such protection is available, and will be presented later.

Table IV shows the extra reducing groups liberated in the mixed conversions at 65° C. due to the presence of β -amylase, being calculated from Table II. There is no evidence that β -amylase is active after 15 min., but the results shown fall into three series:—

Series (i).—There is further increase with time, this occurring only with low enzyme concentrations, and especially when both α and β are low. In a given time, the additional groups are roughly proportional to the increment of D.P. due to β ; thus, for 15 min. conversions, 1° Lintner of β -amylase activity produces approximately

TABLE IV

REDUCING GROUPS PRODUCED AT 65° C. (149° F.) BY MIXTURES OF α - AND β -AMYLASES, ADDITIONAL TO THOSE PRODUCED BY THE SAME CONCENTRATION OF α -AMYLASE ALONE (cf. TABLE II)

(Results are expressed as apparent maltose (ferricyanide) *per cent.* of starch) °

Relative total concn. of enzymes.	Conversion time (min.).	β : α ratio (copper values).			
		2½: 1	3¼: 1	5: 1	6¼: 1
X1	15	0.7	2.3	3.2	3.9
	30	0.9	3.3	4.2	4.9
	45	1.6	4.1	5.2	5.7
	60	1.5	4.4	5.3	6.2
	120	1.9	5.3	6.1	6.8
	Series	(i)	(i)	(i)	(i)
X5	15	10.0	12.8	21.2	25.5
	30	10.0	13.0	20.7	24.3
	45	10.9	13.2	20.5	23.8
	60	12.1	13.4	20.3	23.5
	120	12.2	13.8	21.1	23.1
	Series	(i)	(i)	(ii)	(iii)
X10	15	24.0	26.5	29.3	31.9
	30	23.1	25.7	27.4	29.7
	45	22.9	25.5	26.8	29.0
	60	23.2	25.6	26.5	28.3
	120	23.0	25.4	26.3	28.1
	Series	(ii)	(ii)	(iii)	(iii)
X20	15	22.4	29.0	31.7	32.6
	30	22.5	27.2	29.4	30.4
	45	22.4	26.6	28.4	29.3
	60	22.5	26.4	28.2	28.6
	120	20.3	24.1	25.5	26.4
	Series	(ii)	(iii)	(iii)	(iii)

2.7 *per cent.* of additional reducing groups for the D.P. _{β} range of 3–10° Lintner; within this range, therefore, the activities of the two enzymes are broadly additive for the ratios investigated. The relationship fails at the lowest D.P. values, doubtless because of enhanced instability at the lowest concentrations; it fails also as the inflecting influence of saturation is approached. Again, in this series, more reducing groups are produced continuously as time is prolonged. This is in accord with the view that β -amylase enhances the stability of α , the effect being most prominent when the α -concentration is low and conditions are not obscured by competition. With higher concentrations of α , the enzyme functions to a large extent for its own protection. It may be pointed out that in the initial stages, 1° Lintner of α -amylase activity produces approximately 26 *per cent.* of reducing groups in 15 min. under the conditions used;

hence the conditions of D.P. determination widely exaggerate the contribution of β -amylase in these low-activity conversions.

Series (iii).—Here there is a decrease in further production with time, the delayed result of competition; it will be recalled that on present views of starch structure a proportion of linkages attackable by α are also attackable by β -amylase. Thus, relatively intense hydrolysis in the first 15 min. will, by decreasing the numbers of available linkages, slow up the later activity of α as compared with what would be achieved if it acted alone throughout. Saturation conditions are being approached.

Series (ii).—The additional groups remain substantially constant throughout in this transitional range, the members of the series concerned occupying appropriate intermediate positions in the Table. Here, both protection and competition factors are operating, producing a balance.

Turning now to the influence of temperature (Tables II and III), the results are quite decisive. The influence of temperature increase from 63 to 67° C. on α -amylase alone is small. Thus, under the conditions represented in Fig. 4, such temperature increase

amylase to temperature in the neighbourhood of 65° C. is in accord with the findings of W. D. Claus (*Cereal Chem.*, 1947, 24, 59; this *Journ.*, 1947, 172).

As appears in Fig. 5, the results of maintaining the β : α ratio constant while decreasing the total enzyme concentration are similar. Thus, a low D.P.—like a low β : α ratio—favours temperature sensitivity, and the effect is greater near 67° C. than it is near 63° C. By way of example, 70 per cent. hydrolysis is achieved at 66° C. with an equivalent D.P. of 28.5° L., or at $\sim 64\frac{1}{2}$ ° C. when the equivalent D.P. is 14° L. That the temperature of an infusion mash is a critical one for starch conversion has long been recognized by practical men, but the results now presented would appear to explain at least in part the differing sensitivities of different malts to temperature change.

Our best thanks are due to Mr. R. Brown, who has carried out under our direction a number of preliminary experiments in connection with this work.

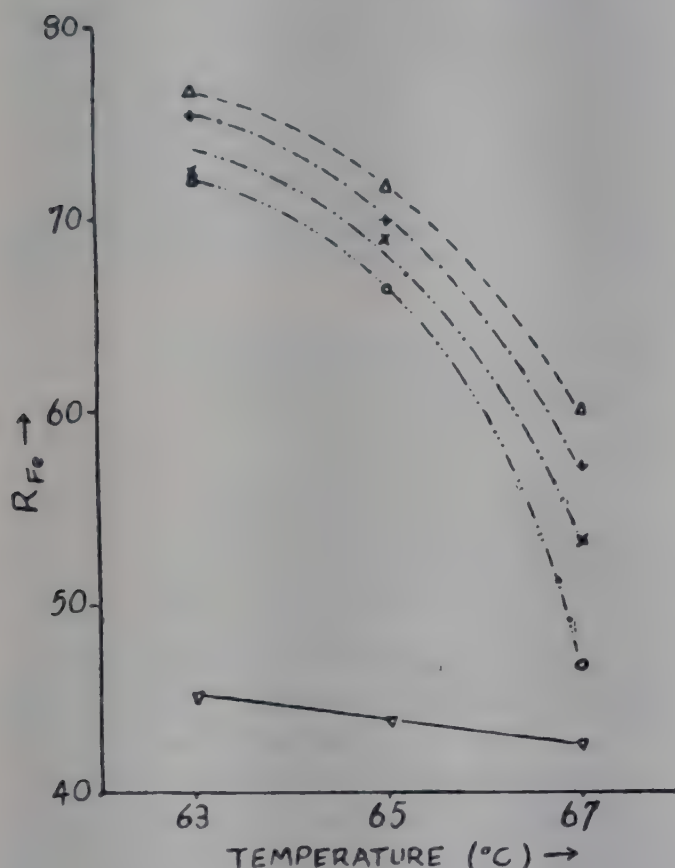


FIG. 4—Influence of temperature and β : α ratio on reducing group production (R_{Fe}). Reading downwards, the ratios are (copper values) 6:1, 5:1, 3:1, 2:1, and α alone. The α concentration throughout is X10. 1 hour conversions.

causes a decrease in reducing group production of about 3 per cent. In mixtures containing β -amylase, however, results are more spectacular; with β : α of 2:1, the fall is 25 per cent., and with 6:1 it is 16 per cent. Thus, for these mixtures, sensitivity to temperature increases as the β : α ratio falls, and the effect of temperature is greater between 65° and 67° than between 63° and 65°. It is of interest to note that, for a constant amount of α -amylase, increase in temperature and decrease in β : α ratio have the same effect on reducing group production; thus, 60 per cent. hydrolysis is achieved at 66° C. with a ratio of 2:1, or at 67° C. with a ratio of 6:1 under the other conditions quoted. This extreme sensitivity of β -

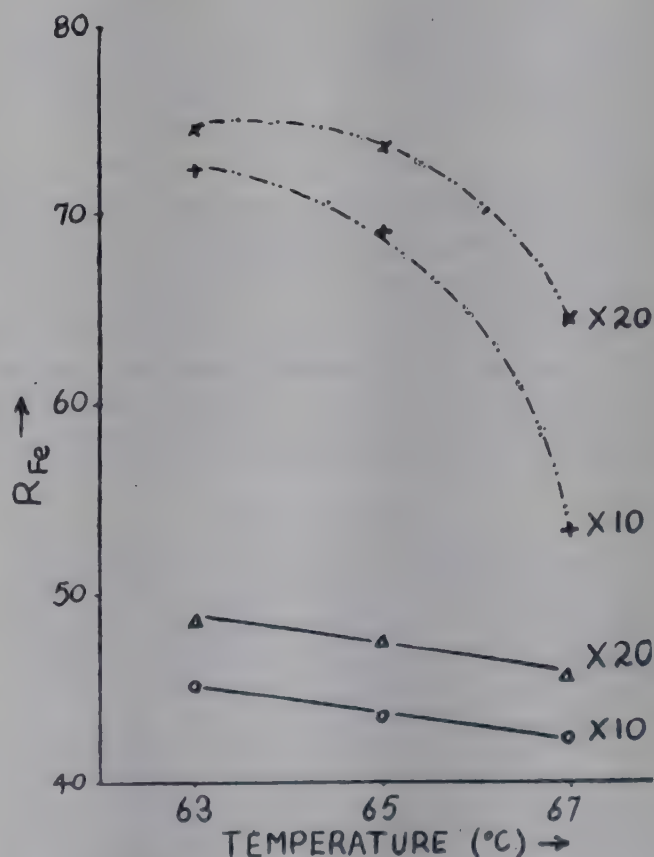


FIG. 5—Influence of temperature and total enzyme concentration on reducing group production (R_{Fe}). Upper curves for β : α = 3:1, lower curves for α alone, 1 hour conversions.

SUMMARY

1. At infusion mashing temperature (e.g., 65° C., 149° F.), β -amylase is active for no more than 15 min. in laboratory starch conversions at p_H 5.4, but its activity during this period is great.

2. Under these conditions, α -amylase is active for prolonged periods, but hydrolysis is extremely slow beyond the inflection point, the level of which varies with the enzyme concentration.

3. A large measure of independence of action of the two enzymes is observed, but at high enzyme concentration competition between them intervenes, whilst with low concentrations of α -amylase there is evidence that β -amylase may exert a measure of protection.

4. Heat sensitivity of mixtures of the two enzymes increases both as the total enzyme concentration decreases and also as the β : α ratio decreases, the sensitivity being due mainly to the behaviour of β -amylase.

5. It should be noted that the above conclusions relate to reducing group production from soluble starch under conditions in which the proportions of enzyme to starch are substantially those to be expected in brewery mashes and where temperature change has been investigated over a relatively narrow range. It is important to note that, in reporting the results of such work, precise conditions of operation must be defined.

Heriot-Watt College, Edinburgh.

February, 1949.

THE COAGULABLE PROTEIN OF SWEET WORT*

By Prof. R. H. HOPKINS, D.Sc., F.R.I.C., and N. J. BERRIDGE, Ph.D.

The protein isolated from sweet wort by Hopkins, Amphlett and Berridge and described by them (this *Journ.*, 1941, 106) has been investigated further. Its most outstanding feature is its susceptibility to calcium salts, in presence of which it is coagulated at much greater rates than in salt-free solution. It also binds calcium. Coagulation is greatly accelerated by the first small quantities of coagulum to be formed, and initial precipitation is facilitated by surface activity and by calcium, which reduce the lag period. The lower the value of p_H the more rapidly does coagulation take place. Maximum turbidity is given at p_H 3.8, but turbidity is reduced by the presence of salts, including those of calcium, over the range of p_H 3-5. Precipitates and coagula contain nucleic acid, the proportions of which tend to increase with fall in p_H . This nucleic acid is responsible for the formation of turbidity and may be associated with the sensitivity of the protein complex to calcium.

INTRODUCTION

IN a previous contribution by Hopkins, Amphlett and Berridge (this *Journ.*, 1941, 106), a description was given of a nucleoprotein isolated by electrodialysis of unhopped malt wort and thereafter purified. Apart from containing nucleic acid, its chief feature was that it was coagulable by heat, appearing to be the only heat coagulable protein in the wort, since the residual protein present in the re-dialysed wort did not form a coagulum on

boiling at suitable values of p_H . It was increasingly soluble on the alkaline side of p_H 5, but fully precipitated at about p_H 3.9, and contained nucleic acid in proportions which varied somewhat according to the conditions of its precipitation.

Investigation of the properties of this protein was interrupted early in 1940 and there seems little prospect of its being resumed in these laboratories. In view of this, and following the recent communication by J. H. St. Johnston (this *Journ.*, 1948, 305), it seemed desirable to set out such results of these pre-war investigations as are available.

* The substance of this paper was communicated to the First International Congress of Biochemistry (Section XII) at Cambridge, 22nd August, 1949.

Sweet wort only was used for the preparation of the protein, it being decided to investigate coagulable malt wort proteins as fully as possible before introducing the further complication of hop tannins, study of the influence of the latter being reserved for subsequent attention. With this important reservation, namely, that other nitrogeneous matter in wort may coagulate or form hazes with hop tannins, our investigations refer to the only protein in wort which is heat coagulable and which, if not removed in this way, would tend to come out of solution as the p_H approaches 3.9. Its properties are reviewed under the headings composition, coagulation, turbidity, etc.

Composition.—Evidence was furnished in 1941 (*loc. cit.*) that the protein under discussion is a nucleoprotein and that colouring matter in the crude preparation is an impurity which is largely eliminated by repeated solution in dilute alkali at p_H 7–8 followed by reprecipitation by acid in the cold (or re-electrodialysis). It may be added that by precipitating about three-quarters of the protein first, the remaining portion could be obtained quite white. Usually crude protein was pink, but reprecipitated samples of it were pale-grey and their solutions yellow.

At 0.2 *per cent.* concentration a solution at p_H 6–7 exhibited a “silkeness,” whilst at p_H 3.7–3.8 it formed distinct flocks. Cruder preparations, obtained by carrying the electrodialysis of the wort further, and often brownish in colour, would disperse less readily to form a suspension in distilled water, but would form a clear solution with alkali at p_H 6 which did not show “silkeness.” A saturated solution at p_H 6 would contain about 40 mgrm. of nitrogen per 100 ml., about 0.28 *per cent.* of the protein.

Coagulation.—During the boiling of the protein solution at suitable acidity, *e.g.*, p_H 5.1, a coagulum is formed in the course of time accompanied by a marked fall in p_H . A precipitate is also formed in the cold if p_H is brought down by addition of acid, and it becomes important to distinguish between this precipitation and true irreversible heat coagulation. This could be done by bringing the liquid in the cold to p_H 8 by addition of sodium hydroxide. A coagulum is not redissolved. The important range of p_H in connection with heat coagulation in wort is 5.6–4.8. If coagulation is brought about at a

reaction below p_H 4.8, the protein is already partly or wholly in suspension before heating, in which case there are visible signs of coagulation in the formation of large flocks. As will be shown below the protein will be present as a suspension at *e.g.*, p_H 5–6 if it has been brought to this reaction by the addition of alkali to a suspension of the pure (isoelectric) protein, whereas it will be a solution if it has been arrived at by cautious acidification of a solution of the protein at p_H 7–8. Such a solution does not precipitate on standing. Over the range of p_H 5–6 it is therefore necessary to investigate the coagulation both of solutions and suspensions.

Of the conditions which affect coagulation, the p_H and the concentration and type of salt present are the most outstanding. Differences in behaviour between protein solutions heated in pyrex and ordinary glassware are probably due either to metallic ions derived from the glass or to a reaction occurring at the surface of the softer glass. For instance, at a p_H of about 6 a protein solution heated in an ordinary soda-glass vessel on a boiling water-bath will coagulate in about two hours, while under similar conditions in a vessel of pyrex glass coagulation will not be brought about in this time. Pyrex glassware was used in the experimental work to be described.

EXPERIMENTAL

Influence of p_H .—A well-washed protein suspension containing about 0.15 *per cent.* of protein was divided into portions of about 12 ml. in pyrex tubes, each portion being adjusted to a different p_H between 4 and 6. Only small volumes of fairly concentrated reagents were used, so that the volume change and therefore the change in the concentration of the protein would be negligible. Exactly 10 ml. of each sample were then pipetted into fresh tubes. The tubes were heated in a boiling water-bath for 15 min. with bulb stoppers to prevent evaporation, cooled, and the p_H values again measured. In each case the glass electrode was washed with distilled water and the washings were added to the appropriate tube in order to avoid loss of protein and to allow estimation of the coagulum on the same sample. A drop of 0.5 N caustic soda was then added to each tube. It was ascertained that this treatment brought the p_H to about 8, so that all the native protein would be in solution, and the alkali would probably have no hydrolytic

effect on the coagulum. The tubes were then centrifuged and the nitrogen in the coagula determined. The results are shown in Table I.

TABLE I
EFFECT OF p_H ON THE COAGULATION OF A
SUSPENSION OF WORT PROTEIN.

p_H (final).	Coagulated nitrogen.	
	mgram. per 10 ml.	Percentage of total nitrogen.
3.30	1.64	73
4.00	1.60	72
4.70	0.95	43
4.88	0.77	35
5.81	trace	<1

As will be shown later, unless the solution is buffered (thus introducing the influences of other ions than hydrogen ions), a considerable change in p_H (about 0.3 in the above case) occurs during coagulation. The values of p_H given in Table I are those of the liquid after boiling.

The previous experiment was repeated with a different preparation of the protein and on a larger scale, but the results are expressed (Table II) *per* 10 ml. of suspension. Boiling was on a glycerine bath for 1 hour under reflux condenser. Numbers 4-6 (Table II) contained acetate buffer (0.015 N) to stabilize p_H .

TABLE II
EFFECT OF p_H ON THE COAGULATION OF A
SUSPENSION OF WORT PROTEIN.

No.	p_H		Coagulable nitrogen, mgram. per 10 ml.
	before boiling.	after boiling.	
1	4.84	4.63	1.6
2	5.34	4.82	1.4
3	5.50	5.09	1.15
4	4.88	4.83	1.15
5	5.29	5.23	1.0
6	5.62	5.52	0.9

From Tables I, II, III it is clear that, between p_H 6 and 4 the more acid the medium up to p_H 4, the more rapidly coagulation takes place. The protein is quite insoluble at p_H 3.7-3.9 so that full and optimal coagulation takes place at that reaction. The influence of p_H on rate of coagulation is also dealt with incidentally in the next section.

Influence of Calcium.—The influence of calcium ions is very remarkable. Solutions at p_H values too high for coagulation in the absence of calcium will coagulate readily when calcium is present in concentrations as low as N/1,000. Coagulation will even occur at temperatures below 100° C., though not so readily, as the following experiment shows.

Portions of a protein solution containing 0.005 N CaCl_2 (equivalent to 20 grains *per* gallon) were adjusted to the p_H values given in Table III and the temperature was raised in a water bath. The temperature at which coagulation took place was noted in each case. The solutions were no longer clear after addition of calcium, but the incidence of coagulation was unmistakable; the cloudy solutions changed sharply to suspensions of coarse particles in a clear liquid.

TABLE III
COAGULATION TEMPERATURE IN PRESENCE
OF 0.005 N CALCIUM CHLORIDE.

p_H	Temperature of coagulation, ° C.
4.29	32
5.28	37
5.42	40
5.80	50
6.16	64

Again coagulation takes place more readily as p_H falls. In experiments such as this it was found that most of the protein was coagulated at 50° C. and p_H 5.5 within the first five min. of the addition of calcium to the solution at 50° C. The remarkable effect of calcium is shown in a striking manner by the low temperatures at which the protein coagulated. This is comparable with the case of edestin, which is denatured by standing in the cold with calcium chloride solution. A parallel phenomenon is the well-known sensitivity to calcium ions of proteins involved in the clotting of milk when treated with rennet.

The next experiment extended the investigation to the influence of calcium in the presence of various buffers. The initial p_H was 5.4 throughout with heating for 150 min. at 100° C. The solutions contained buffers (as stated in Table IV) of 0.008 M; CaCl_2 , where added, was 0.0004 M. The quantity of protein in the reaction mixtures corresponded to about 1 mgram. nitrogen *per* 10 ml., or the same as the coagulable nitrogen in 10 ml. of malt wort of sp. gr. 1075.

TABLE IV

EFFECT OF VARIOUS BUFFERS AND OF CALCIUM ON THE COAGULATION OF THE WORT PROTEIN.

Buffer.	Time required for first appearance of coagulum (min.).	p_H filtrate.	Coagulation, percentage of maximum.
None	129	5.01	98
„ + CaCl_2 ..	5	4.76	100
Citrate	138	5.38	27
„ + CaCl_2 ..	135	5.38	40
Phosphate ..	31	5.30	84
„ + CaCl_2 ..	18	5.28	80
Acetate	—	5.44	8
„ + CaCl_2 ..	78	5.36	69
K_2SO_4	12	5.30	89

It will be seen that:—

(a) Calcium accelerated the onset of coagulation, but did not greatly affect the result after 150 min. boiling, except where acetate was used as buffer.

(b) Acetic acid being volatile, a little was lost during boiling, p_H receded, and coagulation in the absence of calcium never effectively commenced.

(c) The final percentage coagulated was the greater the lower the final value of p_H . But, as will be seen later, the final p_H arrived at is in its turn influenced by the amount of coagulation effected up to the time when boiling is stopped.

(d) Citrate exercised a protective influence, which countered that of the calcium (by removal of calcium ions to form an unionized complex), a phenomenon well known in connection with other proteins.

(e) Although, in the absence of buffer, the visible onset of coagulation was delayed until 129 min., nevertheless it was almost complete after 150 min., there being nothing to check a rapid fall in p_H .

(f) K_2SO_4 , although at 10 times the normality of the CaCl_2 , initiated coagulation more slowly than the CaCl_2 .

A most important conclusion to be drawn from this experiment is that so much depends on the time when the heating is checked, especially if this is soon after the time of the onset of coagulation. It appears in the case of citrate buffer with added calcium that coagulation started so late that it could not

proceed far before the end of the experimental time. Nevertheless, it had 3 min. start of the citrate without calcium and coagulation proceeded 13 *per cent.* further. These and other observations drew attention to a factor which needed to be brought under control, namely the “touching off” of the coagulation. Once this has occurred, coagulation is usually rapid.

When the protein solution at, *e.g.*, p_H 5.4 is heated in a covered pyrex tube by immersion in boiling water, but without shaking, it may be observed that, in time, a skin forms on the surface of the liquid. This may be due to surface evaporation (a little vapour will escape and a little will condense on the cool parts of the tube near the upper end), or to surface activity and denaturation. As the heating continues the thickness of the skin increases till it begins to sink, even if the tube is undisturbed. Usually the skin breaks up and the particles become distributed by convection currents throughout the liquid. Within a few minutes of this happening the coagulation of the protein as a whole has proceeded far enough for the flocks to be easily discernible, and in a few minutes they are large enough to collect at the bottom of tube.

The observation of these phenomena led to the idea that the presence of solid protein facilitated the coagulation, and that this solid was provided in the case of the solution by changes at the surface of the liquid. In order to test whether this was so, a protein solution was heated in a vessel so made that a part of the solution could not come into contact with the liquid-air interface. Such a vessel was made simply by bending a piece of pyrex tubing in the shape of a car crank-handle, one end being closed. The centre limb hindered convection currents so that the liquid in the closed (lower) limb was virtually isolated. The tube was boiled thoroughly with several changes of distilled water to make sure that all traces of salts likely to cause coagulation had been removed. The heating had to be carried out in a steam bath, with other precautions to prevent bubbles of either air or water vapour from forming in the lower (closed) limb. Under these conditions a solution between the p_H values of 5.4 and 6 could be heated in steam for three to six hours according to the actual value of the p_H , without coagulation taking place. In all cases where another portion of the same

solution was heated under the same conditions, but in an ordinary tube, coagulation occurred in less than half the time.

It is interesting, and of possible theoretical significance, that what appeared to be small fibres of denatured protein were sometimes formed. If the solution was very dilute, of the order of one-hundredth of the concentration usually used, the very small amount of coagulum formed by heating was in the form of short threads. Threads of about 1 cm. long were obtained from a solution of the usual concentration by heating at a p_H of about 6 on the water bath for about 4 hours. Denaturation began at the surface, and extended into the solution, causing the threads to hang from the surface. Thus, as long as the concentration of molecules actually undergoing denaturation is kept low, either by diluting solutions at the usual p_H values near 5, or by raising the p_H of solutions of the usual concentration to approximately 6, threads are formed during the heating of solutions of wort protein. The process is somewhat similar to crystallization, the threads corresponding to crystals, if not actually consisting of crystals, and being only obtainable as with inorganic solutions, if the solution is just above saturation point. The analogy to crystallization even extends to the need for a "starter" crystal in some supersaturated solutions and the help to coagulation given by solid protein, although it would be going too far to say that the causes are similar in each case.

Influence of Solid Wort Protein on the Coagulation of the Solution.—The experiments recorded above led to investigations on the influence of added solid protein on the progress of coagulation of a heated solution. A solution

to which was added denatured solid protein equal to one-seventh of its protein content, was heated in an open tube on a boiling water-bath and portions withdrawn at suitable intervals for analysis. In recording coagulated protein results (Table V) allowance has been made for that which was added. A parallel experiment was run with the pure solution.

In this and other similar experiments the presence of denatured protein considerably lessened the lag stage. Indeed, there is no evidence of any lag when denatured protein was added. The native protein exercised a similar, though perhaps less marked, effect.

The kinetics of the reaction are of interest. The residual or uncoagulated nitrogen plotted against time (after the lag phase) yields a hyperbola and its logarithm a straight line. In the case of pure solutions, however, there are breaks in the graphs corresponding to the "touching off" of the coagulation by the first coagulum to be formed. It is not proposed to undertake here a theoretical discussion of the kinetics of these coagulations, but they are consistent with the classical work of Chick and Martin on albumin coagulation (*J. Physiol.*, 1910, 40, 4040). Similar kinetics were obtained in the presence of added calcium, sodium and potassium chlorides. In the case of calcium the striking reduction in the lag phase was confirmed.

Reactions with Electrolytes in the Cold: Electrometric Titration.—Starting with a suspension of precipitated and washed protein at $p_H \sim 4$ and titrating with NaOH, there was a considerable time lag before p_H equilibrium was attained after the respective additions. The protein suspension did not show signs of clearing until p_H 6 was reached, nor did it

TABLE V
EFFECT OF DENATURED WORT PROTEIN ON RATE OF COAGULATION
(original nitrogen in solution approx. 5.5 mgrm. per 10 ml.)

Pure Sol.			Solution plus denatured protein.		
Time (min.).	p_H	Coagulum: (mgrm. N per 10 ml.).	Time (min.)	p_H	Coagulum: (mgrm. N per 10 ml.)
0	5.38	—	0	5.28	—
15	5.35	—	13	5.30	2.52
25	5.37	0.95	18	5.23	3.30
35	5.33	1.39	25	5.22	3.92
61	5.30	3.70	35	5.18	4.37
79	5.20	4.03	—	—	—
106	5.00	4.53	—	—	—

pass into a clear solution until p_H 7. On the other hand, a solution at p_H 7 could be titrated with standard HCl as far as p_H 4.8 before precipitation set in. Both the suspensions between p_H 5 and 7 in the former case and the solutions at the same values of p_H in the latter were stable for days at laboratory temperature, except that the suspensions fell in p_H value on standing, although protected from atmospheric carbon dioxide. The electrometric titration data are not quoted, but it may be stated that the back titration from p_H 7 to p_H 4 yielded practically a straight line, when p_H was plotted against added acid. The results show in a remarkable way how slow protein suspensions (and possibly solutions, too), can be in reaching ionic equilibrium. A similar slowness sometimes makes the adjustment of the p_H of bacteriological media difficult, or even leads to false results.

Turbidity: Influence of p_H .—Small quantities of acid or alkali were added to a suspension of the protein of suitable turbidity and the p_H and turbidity (Zeiss-Pulfrich nephelometer) were measured. Results are shown in Table VI and Fig. 1.

TABLE VI
TURBIDITY OF DILUTE SUSPENSIONS.
(Turbidity Units 100/nephelometer reading.)

(a) Hydrochloric acid. (b) Sodium hydroxide.			(c) Phosphoric acid. (d) Calcium hydroxide.		
	p_H	Turbidity.		p_H	Turbidity.
(a)	2.79	3.8	(c)	2.98	4.0
	3.10	4.7		3.22	5.3
	3.32	5.6		3.63	5.9
	3.60	6.7		3.75	6.7
	3.76	6.9		3.86	5.9
	3.87	6.7		4.18	5.0
	4.12	5.3		—	—
(b)	4.07	5.0	(d)	4.13	4.8
	4.36	4.4		4.11	4.4
	4.64	3.4		4.65	2.4
	5.64	1.03		5.62	1.4

There seems to be a maximum turbidity at p_H 3.8. The iso-electric point is presumably near p_H 3.8–3.9.

Turbidity: Influence of Salts.—The general effect of salts was to diminish the turbidity of the suspension between p_H 3 and 5 and especially near the iso-electric point, almost certainly by altering the degree of dispersion.

Fixation of Calcium.—Evidence was furnished by Hopkins and Amphlett (this *Journ.*, 1939, 365) that not all of the calcium in sweet wort is ionized and there were indications that some was combined with wort proteins, although the possibility of union with phytate was not absent from the authors' considerations. It was decided to test the fixation of calcium by this wort protein. First, addition of $CaCl_2$ up to 0.02 N to a suspension at p_H 5.5 followed by a short stand and analysis of sediment and clear portion, indicated that some of the added calcium was bound to the solid protein. At the same time it was noted

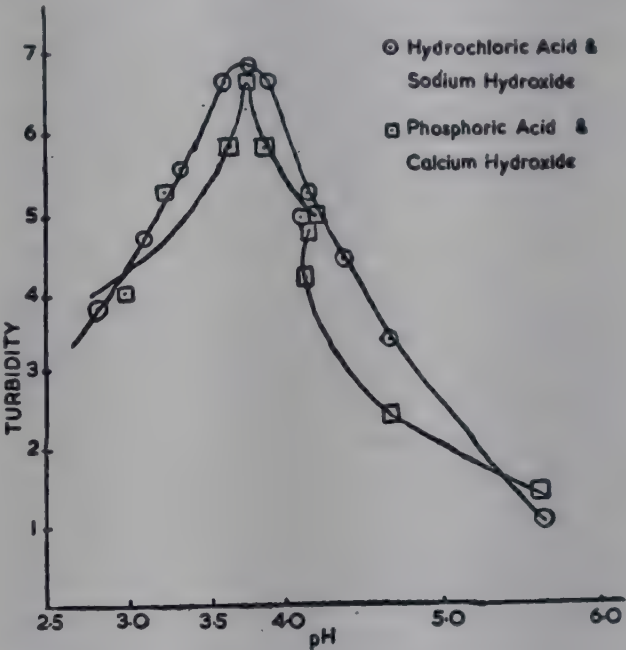


Fig. 1.—Influence of p_H on turbidity.

that the addition of the $CaCl_2$ to the suspension brought about an appreciable fall in p_H , and that this occurred also even if the original suspension was near or below the iso-electric point. The explanation furnished by Hopkins and Amphlett will serve, namely, that the calcium ions combined with ionized carboxyl groups (or possibly nucleic acid in this case), thus inducing dissociation of others with equivalent liberation of hydrogen ions. On the assumption that this liberation is equivalent to the added calcium it is possible to determine the concentration of ionized calcium in the treated solution.

Addition of $CaCl_2$ up to 0.005 N or 0.01 N to a solution at p_H 6 or thereabouts gave a turbid solution, and the p_H fell, but most of the protein was still present in solution. 0.01 N NaOH was then added until the original

value of p_H was restored. From the titration values the equivalent of bound calcium was calculated on the assumption that Ca^{++} liberates $2H^+$. Using the same solutions the principle of the Donnan Equilibrium was applied to the determination of ionized calcium (*cf.* Hopkins and Amphlett, *loc. cit.*) using a collodion membrane. The system was rocked for 24 hours to attain equilibrium. This led to the results of Table VII.

TABLE VII
BINDING OF CALCIUM BY PROTEIN.

p_H suspension.	Ionized calcium, <i>per cent.</i> of total calcium.	
	From Donnan Equilibrium.	Calculated from acidification.
6.20	79	89
5.56	67	67
5.58	89	86

The general agreement in the results obtained by the two methods supports the hypothesis that calcium combines with the protein liberating hydrogen ions.

Suspensions ranging from p_H 6 to p_H 2.7 initially, and solutions from p_H 6 to p_H 5.13 were titrated with $CaCl_2$, a fall in p_H being invariably observed, rapidly with the first incremental addition, but dying away smoothly with later additions.

Results of titrating a protein solution with (a) 0.01 N $CaCl_2$; (b) 0.01 N KCl, using a microburette, are shown in Table VIII.

TABLE VIII
INFLUENCE OF SALTS ON p_H OF PROTEIN SOLUTIONS
IN THE COLD.

KCl.		$CaCl_2$.	
Volume added, ml.	p_H	Volume added, ml.	p_H
0.0	5.34	0.0	5.26
0.23	5.25	0.22	5.06
0.47	5.24	0.43	4.94
0.72	5.21	0.67	4.85
1.09	5.20	0.97	4.80
1.50	5.20	1.21	4.79

Whilst $CaCl_2$ had a marked effect in acidifying the solution, the influence of the KCl was small.

DISCUSSION

The results detailed above may be summarized as follows:—

The protein exhibits minimum solubility and maximum turbidity in aqueous solution at about p_H 3.8. The general effect of salts is to diminish the turbidity between p_H 3 and 5. It is possible for the protein to exist as a clear solution on the alkaline side of p_H 5 and to remain as such for days in the cold and, provided that precautions are taken, it will remain clear for hours at 100° C. On heating for a sufficient time at p_H 5–6 the solution coagulates and p_H falls. The coagulum is insoluble at p_H 8 in the cold. It differs from the precipitate formed when the solution is acidified; the latter precipitate can be redissolved at p_H 6–7 and is reversible. The first coagulum to appear is at the liquid-air interface and its separation is apparently assisted by surface activity. Coagulation is very greatly accelerated either by added coagulum or by undenatured precipitate or merely by the first coagulum to be formed in the heated solution. Coagulation takes place faster the more acid the p_H to a maximum at about p_H 3.8.

Calcium ions have a remarkable influence on the protein. Firstly, in their absence, the solution can be heated under suitable conditions to 100° C. for four hours or more without coagulation, whereas the presence of N/200 $CaCl_2$ enables the protein to coagulate as soon as the temperature rises to 50° C. The effect is an exceedingly great acceleration of coagulation. Secondly, the protein can bind calcium with a stoichiometrically equivalent fall in p_H . The corresponding influences of Na and K ions are very small. The kinetics of coagulation of the solution are in general agreement with those on record for egg albumin.

The outstanding feature of the protein is its remarkable susceptibility to calcium salts. In the absence of salts coagulation of the protein at p_H 5–6 is quite slow, even at boiling, whereas the presence of calcium salts in concentrations of the order that would be found in copper worts is sufficient to bring about rapid coagulation even at much lower temperatures. In this connection it must be remembered that coagulation goes on at enormously greater rates with rise of temperature of only a few degrees.

It is known also that calcium combines

with the protein and makes the solutions milky at ordinary temperatures. This occurs with the pure protein at p_H 5-6, but there is no precipitation in the ordinary sense. Apparently calcium does not precipitate it (or only to a small extent) in sweet wort, otherwise the protein would be precipitated as soon as it passes into solution, *i.e.*, during mashing, and would never appear in the sweet wort. It must be remembered that there is calcium in malt, some of which passes into wort made with distilled water. The influence of calcium is in keeping with the precipitability of protein-nucleic acid complexes noted by Sevag and Smolens (*J. Biol. Chem.*, 1941, **140**, 844), although these authors effected their precipitations with 0.2 M $CaCl_2$. This precipitation may be a property associated with the presence of nucleic acid.

The association of the protein with calcium is a function of the latter in brewing which was hinted at by Hopkins and Amphlett (*loc. cit.*). It is, of course, well known that the calcium ion promotes acidification of mash tun and copper worts by its interaction with phytin and inorganic phosphates. In addition to this we now have its combination with this protein, probably the nucleic acid moiety, leading to fall in p_H and greatly speeding up coagulation of the protein. The fall in p_H of wort due to this cause must be exceedingly small owing to the very small amount of protein involved, but the effect on coagulation is of great moment.

It has not been investigated whether magnesium has a similar effect, *i.e.*, in greatly sensitizing the protein to coagulation. But it can be stated that magnesium and sodium, as well as calcium salts, influence the protein in suspension. Suspensions in the cold are easily flocculated by traces of mineral salts, particularly near the iso-electric point, p_H 3.8. Any haze or fine turbidity that might develop in beer from the presence of any of this protein which had not been fully removed as copper break would be made coarser or be flocculated by mineral salts present.

Longsworth and MacInnes (*J. gen. Physiol.* 1942, **25**, 507) by means of electrophoretic experiments, furnished evidence that a mixture of egg albumin and yeast nucleic acid behaved near its iso-electric point as if the two were loosely and reversibly combined, and on the acid side as if firmly combined inasmuch as a compound was

partly precipitated. Elsewhere the nucleic acid migrated as if it bore a negative charge.

On the basis of all the information available the nucleoprotein (or protein nucleate) may be pictured as follows. The true protein moiety is iso-electric probably over a wide range of p_H between about 5.5 and 6 or more. Not until p_H is brought to below 5 does it acquire a sufficient positive charge to unite at all firmly with the strongly negatively charged nucleic acid ions. At p_H 4.8 the aggregates formed still bear a prevaillingly negative charge and remain somewhat dispersed, precipitation being slight. At p_H 3.8-3.9 mutual discharge and precipitation are complete. The relative proportions of nucleic acid and of protein would tend to vary with conditions of precipitation, the lower the p_H the more nucleic acid present in the precipitate. Results do actually show an increase in P content. At such reactions as, *e.g.*, p_H 3.8-4.8 inorganic salt kations assist flocculation of the fine suspension whilst anions assist on the acid side of p_H 3.9. At p_H 5-5.5, as in copper worts, the protein-nucleic acid (loosely bound) complex bears a negative charge and is soluble and dispersed. Calcium ions help to precipitate it by virtue of their positive charge. In this way they also assist the flocculation of the denatured protein, but their activity in catalysing its previous denaturation may not be simply explained. However, the action of calcium ions on other phosphorus-containing proteins, already noted, suggests that a special affinity exists between the calcium ion in particular and nucleic acid or phosphoric acid derivatives. Altogether nucleic acid in its relations with proteins plays an important precipitating rôle in wort and the brewer's object must be to eliminate it.

The accumulated evidence also confirms the well-known importance of surface activity in coagulation, namely that a break will be improved by a vigorous boil. The coagulation of this protein (as of others) starts at the surface of the liquid and, once started, is rapidly extended. Then the starting of coagulation is assisted by the presence of many bubbles to increase surface activity, by a low p_H and by calcium salts. Once started it "touches off" the further coagulation. The lower the p_H the more complete this is likely to be.

The recent paper of St. Johnston (*loc. cit.*)

makes reference to this nucleoprotein which is identified by the author with his "protein O." The latter is described as being an oxidation product of another fraction which is provisionally termed "protein C." Our nucleoprotein has always yielded analyses in which not less than 90 *per cent.*, sometimes 100 *per cent.*, of the dry matter is accounted for as protein + nucleic acid. On the other hand, St. Johnston's O and C fractions usually contained only from 30 to 40 *per cent.* of protein matter, the rest being carbohydrate, mineral matter, *etc.* His preparations were obtained by the method of ammonium sulphate fractionation of wort. Such products are liable to be heavily contaminated, which is one reason why the technique has not been persevered with here—so far as wort is concerned. It therefore becomes doubtful whether a property ascribed to "protein O," for example, the colouring matter which developed on oxidation, is not due to one or more of the concomitant impurities.

However, there seems little doubt that both protein O and protein C contain nucleic acid and that the former is easily transformed into protein C. Indeed, apart from their considerable impurities the two proteins appear to be identical, the matter reduced by bisulphite being presumably impurity, not protein. No doubt oxygen has an important function, as shown by evidence furnished by the author, but it must function directly with other matters than the protein, presumably tannins and cognate substances. St. Johnston often used hopped wort, which contains hop tannins in addition to the smaller amount of malt tannin present in sweet wort.

A further point arises regarding these proteins and is dealt with by St. Johnston in a paragraph headed "*Relationship between Protein C and Protein O,*" namely the transformation of protein O into protein C and the coagulation of the latter at p_H 6. This was effected in the presence of *calcium bisulphite*. It seems to us that it was the calcium ions which were responsible for a protein, not previously coagulable at p_H 6, now being coagulated. Also the reduction by bisulphite concerned coloured impurity and was fortuitous. Whilst bisulphite has been known to react with certain sulphur-containing proteins (*e.g.*, those of wool) and to some extent reversibly, it may well be doubted whether by this means alone such a remarkable change in protein properties is effected

as is indicated by the transformation from protein O to protein C.

Certain of the properties of "protein C" do not seem easy to reconcile with those of our nucleoprotein, for instance, its stability between p_H 4 and 5. However, in certain circumstances, *e.g.*, most of the nucleic acid having been eliminated by its precipitation in "protein O" (the nucleoprotein) at p_H 3.9, the protein might have a positive charge between p_H 4 and 5 and would in such circumstances be stable (even in the presence of calcium ions). On bringing back towards p_H 6 the positive charge would disappear and precipitation could occur. This may account for certain properties of the "protein C" of St. Johnston.

GENERAL CONCLUSIONS

Without a full investigation of the influence of hop constituents, particularly tannin, on wort proteins and mineral matters it is impossible to state what constituents take part in copper break and haze formation in beer. Here, the properties of the coagulable protein of sweet wort can only be discussed without reference to the complications of added tannin, *etc.* It is, of course, well known that a break can be secured before the addition of hops if this addition should be delayed. It may well be that the coagulable protein is coagulated irrespective of the presence of hops, and that the tannin from the latter, as it is progressively extracted during boiling, interacts only with other wort proteins, peptones, *etc.*

Turning to the properties of the coagulable protein as they have been reported above, it is seen that it is most insoluble at p_H 3.8, that is to say on the acid side of the p_H of beer. Further, it is only imperfectly coagulated at p_H 5.2–5.4 in pure solution and that after some delay. However, an outstanding feature of this protein is its sensitivity to calcium. Coagulation readily takes place at p_H 5.4, even below boiling temperature in the presence of 20 grains *per* gallon of calcium chloride or sulphate. Furthermore, the protein is flocculated by calcium into a coarse break. It seems that the presence of calcium salts in the brewing liquor, added to the smaller contribution from the malt, speeds up and otherwise improves protein break in the copper. The protein thereby removed is one which, if left dissolved, would become increasingly insoluble as p_H fell in value during

fermentation and storage. Even small quantities not removed may contribute to protein turbidity or haze in the beer. Here again, the influence of mineral salts would be beneficial in that flocculation of the protein would be promoted with improved chances of its removal. At p_H 4 and thereabouts, the turbidity of a suspension of the protein was decreased by added salts.

The susceptibility of the pure protein to surface activity is also a point of practical interest. Clearly its coagulation is promoted by a violent boil which creates much surface between wort and bubbles, at which surfaces

the first films of insoluble coagulum would be formed. Once formed, the insoluble protein "touches off" the coagulation of the remainder, at least to any limit that may be imposed by p_H and wort composition.

One of us (N. J. B.) was indebted to the Department of Scientific and Industrial Research for a grant which enabled this work to be performed.

British School of Malting and Brewing,
University of Birmingham.

5th February, 1949.

***STREPTOCOCCUS MUCILAGINOSUS* KULKA, COSBIE AND WALKER** (*Spec. nov.*)

By DORA KULKA, Ph.D., A. J. CURTIN COSBIE, B.Sc., A.R.I.C., and
T. K. WALKER, D.Sc., F.R.I.C.

Particulars are given of the isolation, characteristics and classification of a catalase-negative, tetrad-forming streptococcus, which was obtained from a ropy beer. The most distinctive feature of this organism is its propensity to render beer extremely viscous, not only under anaerobic but also under aerobic conditions, and this propensity has persisted over a period of five years, during which the organism has been cultivated in the laboratory. In its principal characteristics it differs so markedly from those beer streptococci which have been described previously, and indeed, from other non-pathogenic streptococci concerning which particulars have been published, that it is to be regarded as a new species. The habit of producing a very viscous condition both in bottled beer and in beer in cask renders it particularly virulent as a beer disease organism.

INTRODUCTION

MICRO-ORGANISMS belonging to the group of the beer streptococci have usually proved more difficult to investigate than the majority of beer spoilage bacteria, and it is perhaps due to the difficulties attendant upon the isolation of the beer streptococci that so few members of this group have been described adequately in the literature. The principal difficulties encountered when working with these organisms are related to the fact that the individual cells are not always easy to identify even in the stained condition, and particularly is this so when they have to be sought for in products which are in an advanced state of deterioration. The individual cells are not only very small and often take the stain irregularly, but it frequently happens that they are unevenly distributed throughout the spoiled material,

and further, they are often numerically insignificant in relation to the damage which has been done. Hence in searching for the source of the trouble such small cells may easily escape notice. For this reason it is often necessary to rely on the use of enrichment methods using special media in order to isolate such organisms. Further, slime-forming species are difficult to obtain in pure culture for purely mechanical reasons in that their cells sometimes adhere to those of other species of micro-organisms.

Beer streptococci in company with the rod-shaped lactobacilli are fastidious in their nitrogen requirements and often it is not easy to maintain them in the state in which they are isolated. In fact the maintenance of some strains depends on ability to determine the nature of some particular

constituent or constituents which it is essential should be present in the medium used for their propagation.

It might, therefore, be helpful to describe the technique which was devised to isolate the newly investigated rope-forming coccus and further to describe media which, in the course of several years, have been used to maintain unchanged a collection of *Lactobacillus* species from beer, including the rope-forming coccus which is the subject of the present communication.

EXPERIMENTAL

Isolation of the Streptococcus

A small volume of the ropy beer was inoculated into unhopped wort. A fermentation ensued owing to the presence of living yeast cells, and development of the coccus followed the fermentation brought about by the yeast. Some days after the completion of the fermentation the heavy yeast deposit at the bottom of the tube was examined, but few cells of the coccus could be observed. Those seen were usually small, they were Gram-positive and occurred singly, in diplo-forms and as tetrads. They were always seen adhering to the surface of yeast cells. Plating out, using a series of different media, failed to result in the formation of colonies of the cocci and the plates yielded mainly yeast colonies containing a few cells of streptococci. The use of selective media designed to suppress the growth of the yeast and favour the coccus invariably resulted in no growth whatever. This indicated that the coccus depended upon a nutrient or nutrients which it obtained from the yeast cells. In addition, the microscopical examinations indicated that the cocci were attached to the yeast cells by slime, in such a manner that no separation could be expected from merely shaking the suspended mixture of contiguous cells, in the usual way, in physiological saline. Eventually, the desired separation was effected by use of dilute sodium hydroxide, which dispersed the slime sufficiently to allow the cocci to come apart from the yeast cells to which they had attached themselves. It was found after numerous trials that the concentration of sodium hydroxide which produced the best separation was very close to that which had a pronounced germicidal effect, so great care had to be exercised in its application. Sodium hydroxide used at concentrations between

0.15 grm. and 0.30 grm. per 100 ml. gave good results after shaking for 5 min. at room temperature. Immediately afterwards the suspension was raised to 37° C. and maintained at that temperature for a further period of 5 min. Plating-out was then performed and resulted in the formation of well-separated single-cell colonies of yeast, with the cocci growing separately as tiny colonies only on those plates which had received a heavy inoculation.

Appearance of Single-cell Colonies

These were from 0.5–0.75 mm. in diameter on plates which had more than 100 colonies *per* plate and 1.0–1.5 mm. in diameter on plates with less than 10 colonies *per* plate. The colonies were off-white, opaque, glistening and dome-shaped. When examined under low power they showed an entire margin. The colonies were extremely coherent and of ropy consistency; when touched with the inoculating needle they stretched out in long threads which could not be torn off by further extension. This property rendered troublesome the transference of cells to a new medium.

Microscopical Appearance of the Cells of the Colony.—They appeared as diplococci and were Gram-positive.

Morphological Characters

After aerobic growth for 6 days at 25° C. in a medium consisting of 3 volumes of wort plus 1 volume of yeast-autolysate the cells appeared to be spherical, elongated forms being absent. They were uniformly 0.5 μ in diameter and were arranged in diplo-forms and as tetrads as well as single cells. Spores were not observed and the cells were non-motile. The individual cells were surrounded by small capsules and these could be observed even in young cultures which were highly ropy.

Cultural Characters

In Liquid Media, at 25° C.—With free access to air the organism developed best in the following media:—

- (1) Casein-digest with addition of glucose or of malt extract; the p_H value of such a medium was adjusted to 5.4.
- (2) Yeast extract with added glucose or Pasteur's yeast-water with added glucose.

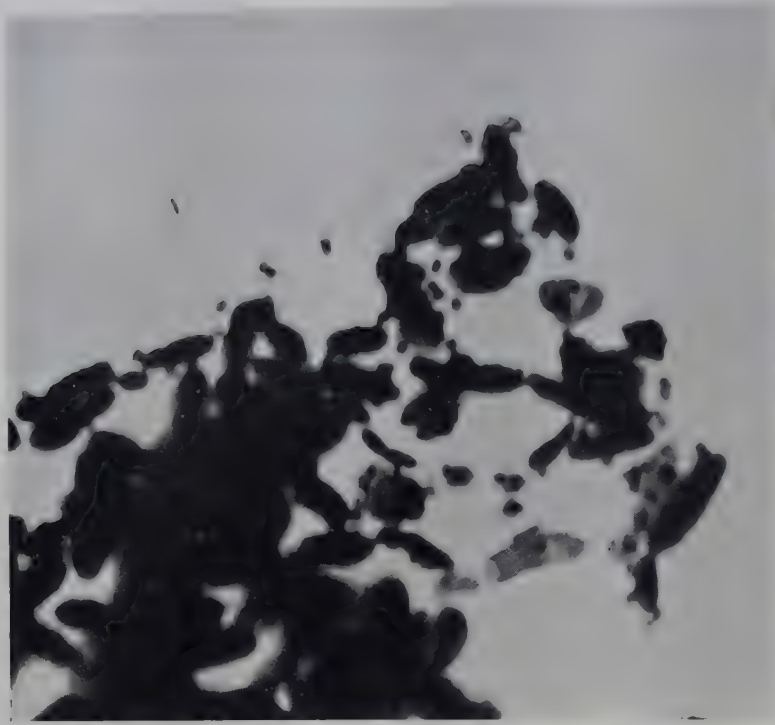


Fig. 1. *Str. mucilaginosus* (X 1500) growing in proximity to yeast.



Fig. 2. *Str. mucilaginosus* on wort-agar slope.



Fig. 3. *Str. mucilaginosus* on glucose-lemco-peptone-agar slope.

- (3) The use of a yeast autolysate medium containing glucose gave rise to very strong growth of the cocci.
- (4) In a culture consisting of 1 volume of yeast autolysate to 3 volumes of wort, the medium, after two days, appeared brilliant but not ropy and a coherent sediment was attached to the bottom of the tube. After four days the medium was opaque and more viscid with extremely slimy, rich, sedimentary growth. No cells were detectable in the supernatant ropy liquid. After 17 days this medium was nearly clear and of normal thin consistency, and had a deposit which was still coherent, but could be detached from the bottom of the tube.

The behaviour of the coccus towards each of a selection of different sugars was studied in a medium consisting of casein double digest. To separate portions of this digest the following were added, in each case in 1 *per cent.* concentration:—L-arabinose, D-xylose, fructose, maltose, sucrose, lactose, raffinose, mannitol and salicin. In every case except that of fructose, the type of growth which ensued was similar to that which had been observed in a yeast autolysate plus wort medium, as described above. The fructose medium, on the contrary, did not become ropy, but yielded a deposit which finally was entirely powdery and not coherent.

In the absence of carbohydrates, variable results were obtained on casein-digest. The casein double digest described by Davis (*Dairy Industry*, 1939, Nos. 9 and 10) gave no growth, but growth occurred on an ordinary commercial casein-digest.

Good growth was obtained in a medium consisting of one volume of Levinthal-bouillon (see below) to one volume of unhopped beer with the addition of 1 *per cent.* of glucose; this is the medium which has been found most suitable generally for maintaining cultures of lactobacilli without deterioration over long periods of time. The new coccus grew poorly in plain wort but, nevertheless, development was typical in this medium. The culture remained clear all the time during incubation, slowly became viscid and gave rise to a coherent sedimentary growth. Liquid lemco-peptone broth fortified with glucose gave rise to a rich growth of the same type as that observed in media containing casein or yeast extract. In the absence of glucose the results

varied with the source of the peptone. Using broths containing different peptones the following results were obtained after 14 days' incubation at 25° C.:—in the presence of Witte peptone or peptone prepared by Evans no growth took place. Difco peptone, on the other hand, gave rise to development of the cocci. In carbonated beer the development of the organism showed the same stages as occurred in the yeast autolysate cultures described above.

On Solid Media.—Giant colonies on a medium consisting of Levinthal-bouillon (Levinthal, *Zentr. Bakt. Parasitenk.*, 1931, 121, 513) plus wort together with 2 *per cent.* of agar after 11 days at 10°–15° C. showed strong development. The growth was richer still, semi-transparent and yellowish after 18 days. At one month the colonies were transparent, "running," slightly ropy and more than 1 cm. in diameter. When examined microscopically, only single cells and diploforms were observed.

Physiological Characters

Oxygen requirement.—Facultative anaerobe, but more tolerant of oxygen than any other member of the group of beer lactic acid bacteria which has been tested in this laboratory.

Optimal Temperature.—25° C. in yeast autolysate-wort. The organism grows at 10° C. and also at 30° C., whereas Mees (*Onderzoekingen Over de Biersarcina*, Thesis, Delft, 1934, abstracted in *Ann. Fermentations*, 1935, 1, 54), and Shimwell and Kirkpatrick (this *Journ.*, 1939, 137) have reported that beer streptococci are checked or suppressed at 30° C. Recently, in the course of the present work, it has been observed that some strains of beer streptococci are suppressed even at 28° C.

Biochemical Characters

Catalase Reaction.—Negative.

Voges-Proskauer Reaction.—Negative.

Diacetyl Test.—Negative. This test was made not only in young highly-ropy cultures in glucose broth, but also in old cultures and in beer infected with the coccus, the method employed being that which involves the nickel dimethylglyoxime reaction used by van Niel (*Biochem. Z.*, 1927, 187, 472). When the coccus is grown in media containing

glucose in the presence of calcium carbonate much lactic acid is produced. The calcium salt of this can be crystallized and the acid identified by the usual tests. The organism is homofermentative and does not give rise to evolution of gas when grown in media containing different sugars.

Acid Formation from Carbohydrates.—Although the organism in casein double digest can utilize different sugars, it is only from glucose, fructose and salicin that it is able to produce acid.

DISCUSSION

The following is a brief summary of the distinguishing characteristics of the species *Streptococcus damnosus* (Claussen) Shimwell and Kirkpatrick, as given by these authors:—Gram-positive, catalase-negative, non-motile, non-sporulating, sometimes encapsulated spheres of variable size, averaging about 1 μ in diameter, occurring in irregular clumps, pairs, tetrads and chains (not in symmetrical cubical packets). Cultural characteristics similar to other saprophytic streptococci of plain type, growth stimulated by yeast extract and malt extract. Gelatin not liquified. Optimum temperature 21°–25° C. Attacks glucose, fructose, maltose and sucrose, does not attack pentoses and lactose. Butter aroma due to diacetyl is a notable feature in its beer and wort cultures, which may also become reddened in colour. Oxygen requirements: facultative anaerobe in carbohydrate media. Optimum p_H value, 5.0 to 6.0. Little or no growth at p_H 7.0 or higher. Produces low p_H values (3.8) in hopped beer. In addition, a variety of this species, known as *Streptococcus damnosus* var. *viscosus*, according to Shimwell and Kirkpatrick exhibits slime formation as a constant character when grown anaerobically in hopped beer saturated with carbon dioxide. When grown in flat beer it produced only the ordinary symptoms of the so-called sarcina-sickness. Further to this information, Shimwell (this *Journ.*, 1948, 237) has now published a communication dealing with ropiness due to tetrad-forming cocci. In this last paper, he states that the strain of *Str. damnosus* var. *viscosus* which has been maintained in cultivation by him since 1939 has now lost entirely its slime-producing propensity, and he proceeds to describe another organism which he designates *Strep-*

tococcus damnosus var. *limosus* Shimwell. This is a tetrad-forming coccus from ropy British bottled pale ale. *Str. damnosus* var. *limosus* Shimwell occurs as spheres (sometimes slightly ovoid), variable in size even in the same culture, averaging about 1 μ , though large cells up to 1.5 μ are sometimes produced. In beer cultures voluminous capsules are present. The organism renders wort extremely ropy and produces ropiness in beer also, though in this case the intensity of slime formation varies with the composition of the beer. In beer, diacetyl is produced also. The optimum temperature range for growth in wort is given as 21°–25° C. (70°–77° F.) and no growth is possible at 30° C. (86° F.) The organism is facultative, producing ropiness both aerobically and anaerobically. In "Difco" yeast extract acid is produced from glucose, fructose, maltose and sucrose, but not from lactose, raffinose, dextrin and starch. The organism produces ropiness (and acid) only in vegetable media such as beer, malt-wort or yeast-extract. In glucose-broth, only a slight slow growth occurs, with a mere trace of acid, the sediment produced being of a white fluffy flocculent type.

These summarized particulars of *Str. damnosus* var. *viscosus* and of *Str. damnosus* var. *limosus* Shimwell have been given in order to permit direct comparison of the principal features of these two organisms with the corresponding features of the coccus which forms the subject of the present communication. In the following paragraphs, reference is made to the features and properties which differentiate our coccus from those strains of *Str. damnosus* which Shimwell has described:—

- (1) The coccus now described does not show a preference for growth in plant extracts such as malt-extract. In plain malt-wort it shows but poor growth, clear and ropy, never turbid. If grown side by side on wort and on Difco peptone broth without sugar, growth is not less in the broth than in the wort; growth is much stronger in broth containing sucrose than it is in wort; *i.e.*, in contrast to the known beer-streptococci the new organism grows better in broth than in wort.
- (2) Unlike other beer streptococci which have been described, the present organism does not form diacetyl or acetyl methyl carbinol.

- (3) The newly described organism in casein double digest gives rise to acid only from the glucoside salicin and the sugars glucose and fructose, though in a single case a very little acid was produced from maltose. *Str. damnosus* var. *viscosus* and *Str. damnosus* var. *limosus* form acid from glucose, maltose and sucrose, but not from salicin.
- (4) The individual cells are uniformly $0.5\ \mu$ in diameter in contrast to the cells of the *Str. damnosus* varieties which average $1\ \mu$ and in some cases may be as large as $1.5\ \mu$ in diameter.
- (5) The newly described coccus will grow at 30°C ., whereas other beer streptococci refuse to grow at this temperature and many are suppressed at 28°C .
- (6) According to the descriptions given by Shimwell (this *Journ.*, 1940, 207; *Wallerstein Lab. Commun.*, 1941, 4, 41; this *Journ.*, 1948, 237) the capsules both of the ropy coccus isolated by Davis (*Proc. Soc. Agric. Bact.*, 1936, *Nat. Inst. Res. Dairying* Reprint No. 381) and of *Str. damnosus* var. *limosus* are voluminous, whereas those of our coccus are small.

In view of these differences, it is clear that our organism does not represent a strain of *Str. damnosus*; hence Shimwell's conclusion that *Str. damnosus* var. *limosus* is "identical with the ropy coccus about the existence of which brewing opinion has been unanimous for so long," may perhaps be called into question, for it might equally well be argued that our coccus is identical with this unclassified ropy coccus of the literature.

Classification of the New Organism

The newly described coccus has been shown to belong to the family of *Lactobacteriaceae*, the members of which are characterized as non-spore forming, Gram-positive, catalase-negative organisms (rod-shaped or coccus forms) which require for their development protein degradation products of definite types, together with growth factors from specific sources and which break down carbohydrates to lactic acid as the main (sometimes the sole) product. Further, it falls into Tribe I *Streptococceae* of the *Lactobacteriaceae* (see D. H. Bergey *et al.*, *Manual of Determinative Bacteriology*. Baltimore and London, 6th ed., 1948) and is homofermentative. It could be assigned to

Genus III *Leuconostoc* of tribe *Streptococceae* according to the sub-division of these species made by Bergey *et al.*, though it is not identical with any one of the *Leuconostoc* species listed by Bergey. The latter are heterofermentative and the homofermentative character of the new organism gives it affinities with the organisms of Group C of Genus II *Streptococcus* (of which *Str. lactis* and *Str. cremoris* are well-known members) in the sub-division of the lactic acid-forming streptococci according to Bergey *et al.* The new organism must therefore be regarded as a form intermediate between the organisms of these two groups, and the decision to assign to it this intermediate standing is reinforced by the fact that, unlike the organisms of these groups, it does not display a preference for media containing plant material, but grows equally well on lemco-peptone-glucose broth. Other beer streptococci, for example, *Str. damnosus* and related organisms, which are regarded by Shimwell as intermediate between Genus II and III (above), display a preference for nutrients of plant origin. The new organism differs from the *Str. damnosus* group in this and several other important characteristics, as already described. The new organism possesses features not exhibited by such lactic acid-forming streptococci from fermentation media as have been described in the literature and it cannot be identified with any of the lactic acid-forming cocci described in Bergey's *Manual of Determinative Bacteriology*.

The writers regard their organism as a new species and in view of its ability to render certain media highly viscous, they suggest the designation *Streptococcus mucilaginosus* under which the species is now proposed as new.

STREPTOCOCCUS MUCILAGINOSUS

KULKA, COSBIE AND WALKER

Cells spherical, uniformly $0.5\ \mu$ in diameter, arranged in diplo-forms and as tetrads as well as single cells. Non-motile, no endospores. Gram-positive. Capsules present. Grows well in (a) a casein-digest medium with addition of glucose or of malt-extract, (b) in a yeast-extract or yeast-water medium with addition of glucose, (c) in unhopped beer and (d) in lemco-peptone broth with addition of glucose. Usually the medium remains clear but gradually becomes very

viscous; later the viscosity decreases. Growth weak in plain malt-extract.

Homofermentative. Acid from glucose, fructose and salicin in a casein double digest medium; no acid from other sugars or sugar alcohols. Catalase-negative. Gelatine not liquefied. Facultative anaerobe. Distinctive characters: more tolerant of oxygen than are other beer streptococci. In contrast to the well-known beer streptococci it grows more strongly in lemco-peptone-sucrose broth than in wort. Unlike the majority of beer streptococci it does not form acetyl methyl carbinol or diacetyl.

Optimum temperature 25° but it grows at 10° and also at 30°. The majority of beer streptococci cannot grow at 30°. Isolated from

beer which had become viscous, Manchester, England, 1944.

Sub-cultures from the type culture have been deposited at the National Collection of Type Cultures, Lister Institute, London, at the Department of Industrial Fermentation, University of Birmingham and at the National Institute for Research in Dairying, Shinfield, Reading.

The viscous material produced by *Str. mucilaginosus* is being subjected to chemical examination by one (T. K. W.) of the authors.

College of Technology,

University of Manchester.

16th July, 1949.

ABSTRACTS

I.—MATERIALS AND ANALYSIS

Kenia and Allied Varieties of Malting Barley. H. HUNTER (*Farming*, March, 1949; *J. Incorp. Brewers' Guild*, 1949, **35**, 216-222). Plumage-Archer and Spratt-Archer barleys occupy a prominent place in English agriculture since they rarely fail to produce grain of good malting quality and most of the few failures are due to late sowing, an excessive rate of seeding, or a tendency to harvest the grain before it is fully ripe. During recent years Kenia and Maja varieties and hybrids derived from these have been grown in this country, since they have met with some success on the Continent. Although claims have been made that Kenia grown in this country gives a higher yield than does Spratt-Archer, such statements are not supported by the results of tests on growths of each variety made under strictly comparable conditions over several years. Analyses of the above growths showed that Kenia on an average contained 0.12 *per cent.* more total nitrogen, the 1,000 corn weight was 3.6 gm. lower, and the brewers' extract *per qtr.* was 1.4 lb. lower than that of Spratt-Archer. Divergent opinions are expressed by different maltsters on the merits of these new varieties since some will buy and use those of the Kenia series, whilst others consider them unsuitable for malting. The merits of the Kenia series lie in their shorter and stronger straw, the relatively early period of ripening, and their ability to use large amounts of artificial fertilizers without serious danger of "lodging." These qualities are advantageous when the combine harvester is used. In northern areas where ripening extends into early autumn the relatively earlier maturation of the Kenia series is of considerable value, but the nature of the crop represents a departure from the widely accepted standard of British malting quality, and for this reason other new varieties such as Earl are more acceptable. Several varieties derived from Kenia and Maja exhibit higher malting quality than the original parents, but show a greater susceptibility to smut than do Spratt-Archer and Plumage-Archer; hence care should be exercised when locating the position of such crops in relation to those of native varieties to be used as sources of seed. T. J. W.

Seed Globulins of the Most Common Species of the *Gramineae* and Their Differentiation in the Seed. C. E. DANIELSSON (*Biochem. J.*, 1949, **44**, 387-400). The seed globulins of nine different species of *Gramineae* have been investigated. The globulin fractions

were prepared largely by Quensel's method, in which the ground seeds are extracted overnight at 4° C. (39.2° F.) with M sodium chloride solution at p_H 7.0, the extract fractionally precipitated with solid ammonium sulphate, the fractions dissolved in 0.2 M sodium chloride solution at p_H 7.0, and the solutions dialysed in a cellophane sac against distilled water. The globulin components isolated were studied mainly with the ultracentrifuge. Two well defined components, α and γ , were isolated, the α -globulin being found in wheat and barley and four other species, and the γ -globulin in wheat and barley and six other species. The β and δ components were found only in barley, the δ in very small amounts. The β -globulin (which other work has shown to have some association with the chill haze of beer) is distinctive by containing 1.96 *per cent.* of sulphur compared with 1.65 *per cent.* in the α and γ components, and is very stable towards heat and enzymes. The sedimentation constants found for the four globulins are the same as those given by Quensel (this *Journ.*, 1945, 259). Earlier work has shown that malt contains the same components as barley, and the sedimentation diagrams from barley and malt are identical. If the β and δ components of barley are disregarded, the different species within the *Gramineae* seem to have practically the same seed globulins, the γ component predominating in species that have little reserve protein. The reason for this last fact is shown in a study of the distribution of globulins in wheat and barley seed. In wheat, only γ -globulin was found in the embryo, and all the α and a small amount of γ -globulin was in the residual fraction of the seed. In barley all the γ -globulin was in the embryo, together with a small amount of α -globulin, whilst most of the latter and all the β -globulin was found in the residual fraction. Obviously, in seeds with a low protein content most of it would have to be in the embryo, and therefore the globulin would be mainly of the γ -type. The husk fractions in both wheat and barley contained γ in higher concentration than α -globulin. Attempts have been made to degrade in different ways the purified γ -globulin from wheat embryo, to find whether α -globulin can be obtained as an intermediate product, and so establish a biochemical relationship between the two components. These attempts have given negative results. The molecular weight found for the γ component was 210,000, the difference from Quensel's value (166,000) probably being due to the use of the completely pure substance. The molecular weight of α -globulin was found to be 29,000 (Quensel, 26,000). A. A. D. C.

Reducing Power of Sugars in Carbonate-Buffered Cupric Tartrate Micro Reagent.

L. J. HEIDT, F. W. SOUTHAM, J. D. BENEDICT and M. E. SMITH (*J. Amer. chem. Soc.*, 1949, **71**, 2190-2196). The method of Shaffer *et al.* for the determination of reducing sugars with a cupric tartrate micro-reagent (*J. biol. Chem.*, 1945, **160**, 60) has been improved to give greater reliability and 10 *per cent.* higher yields of cuprous oxide. The reagent consisted of 5.0 gm. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 17.0 gm. Rochelle salt, 25.0 gm. sodium carbonate, 0.7 gm. potassium iodate and 1.0 gm. potassium iodide *per litre*, with enough sodium bicarbonate to bring to p_{H} 9.00. The reaction conditions giving best reproducibility and the greatest practicable yields consisted in heating for 25 min. at 100° C., using a reagent buffered at p_{H} 9.00 and containing 0.06 M tartrate. Reactions were carried out in test tubes containing 0, 1, 2, 3, 4 and 5 ml. aliquots of 0.0016 M sugar solutions, each with 5 ml. of reagent and water to bring the final volume to 10 ml. Yields of cuprous oxide were determined iodimetrically and plotted against sugar concentrations. The plots were linear and reducing powers were evaluated from the slopes of the calculated best straight lines. The average reproducibility of each analysis was 1.2×10^{-7} mole of cuprous oxide. For a range of 14 aldoses and 2 ketoses, higher yields of cuprous oxide were obtained under equivalent conditions from sugars whose neighbouring hydroxyl groups were *trans* than when those groups were *cis*. C. R.

Determination of Malt Extract in the Light of Brewery Requirements.

B. TROLLE (*Wallerstein Lab. Commun.*, 1949, **12**, 127-140). The Congress method of malt extract determination, although generally used, affords results not comparable with large-scale practice. One error arises because the proportion between water and extract solids in the filtered mash differs from the proportion in the entire mash, an effect due to the influence of the spent grains. By considerable dilution of the mash before weighing, this error is reduced but that involved in specific gravity determination is increased. A micro method devised by Windisch employs a mash of 1 gm. of malt with 4 ml. of water; extract is calculated from the weight of dried spent grains, the water involved and the moisture of the malt. Errors involved depend upon lack of knowledge as to the exact quantity of water involved in hydrolysis of the malt. In the double filtrate method of Leyser, the mash is partially filtered, the filtrate weighed and an identical weight of water added to the residue which after further filtration affords a diluted wort. Extract is calculated from the specific gravities of first and second filtrates, and the effect of grain absorption of wort solids is

thereby eliminated. The usefulness of the method depends upon the validity of the assumptions that (a) water retention by spent grains is independent of wort concentration; (b) the water retained by the filter paper need not be measured; (c) the concentrations of the first and second filtrate are the same. The assumptions have been carefully tested and found to be true, and reproducibility of results is readily obtainable. A correction factor applied to the ordinary Congress method gives results approximating to those obtained by the double filtration method.

Using the double filtrate method of extract determination an attempt was made to determine the best method of obtaining maximum extract yield from malt. The results indicated that investigations into the pre-mashing method should be productive of useful results. Pre-mashing is well known to increase yield, but the increase cited in the literature may be improved upon. Mash concentration has little effect upon yield; increasing temperature (65-75° C.) effects a slight reduction, and prolonged infusion a small increase. The addition of small quantities of lactic acid to the mash liquor also causes an increased yield. The upper limit to yield was not established, but pilot brewery experiments indicated a sacrifice of beer flavour in all cases where abnormal methods were used. For laboratory purposes, the author advocates mashing by the same procedure as used in the brewery, followed by double filtration. Distilled water is to be preferred and boiling and hopping do not interfere with the results. A method is appended for the analysis of spent grains.

A. E. W.

Improved Separation of Sugars on the Paper Partition Chromatogram.

M. A. JERMYN and F. A. ISHERWOOD (*Biochem. J.*, 1949, **44**, 402-407). The object of this work was to develop methods for the quantitative analysis of mixtures of the simpler hexoses and pentoses. Solvents previously used for sugars did not give adequate separation of the constituents of such mixtures, which, in paper chromatograms, needed solvents of large partition coefficients, corresponding to an R_f value of about 0.2 for the sugars. Such solvents would have to be of low viscosity for the length of movement involved, and two new solvents have been found to fulfil these conditions and give good separations. They are the 3-component mixtures ethyl acetate-acetic acid-water and ethyl acetate-pyridine-water, in which it is possible to adjust the partition coefficient over a wide range by varying the amount of one of the components. Separation of sugars with such low R_f values would normally need very long papers, but the difficulty was avoided by allowing the solvent to drip off the end of the paper. The apparatus used was similar to that

of Partridge (this *Journ.*, 1949, 247), the main difference being that a small hole was bored through the lid of the chamber immediately above one end of the trough in order to deliver solvent to the trough from a pipette. The hole was normally closed with a tight-fitting bung. By filling the trough without opening the chamber, after the paper and atmosphere had reached equilibrium with the solvent, disturbance of the phases on the paper was avoided. The procedure followed was to add standard spots (3–7 μ l.) of sugar solution to the papers, which when dry were hung from the trough, no solvent being present. Sufficient of the non-aqueous and aqueous phases was now added to the bottom of the chamber to ensure that the papers would reach equilibrium with the solvent, the lid was placed in position and the apparatus left overnight. The next day solvent was added to the trough through the hole in the lid and the bung replaced. The papers were subsequently treated according to Partridge (*loc. cit.*). The temperature was maintained at 20° C. (68° F.) throughout the test. In a mixture containing arabinose, xylose, glucose, galactose, galacturonic acid and mannose, it was possible to detect all the components with certainty, using one or other of the above solvent mixtures. If only a small proportion of one sugar was present it was advantageous to use a concentrated solution, which was possible with these solvents because the distance between the sugar spot centres was 3–5 cm. Quantitative analysis of the sugars on the chromatogram was made by a combination of the methods of Hawthorne (*Nature*, 1947, 160, 714) and Hirst, Jones and Flood (*ibid.*, 86), and the results of analysis of four sugar mixtures are given.

A. A. D. C.

Colorimetric Determination of Fructose in Presence of Glucose. D. T. ENGLIS and J. W. MILES (*Analyt. Chem.*, 1949, 21, 583–584). The method is based on the observation that fructose produces an intense colour in reaction with the Folin-Denis phenol reagent (*J. biol. Chem.*, 1912, 12, 239) whereas glucose gives only a slight colour. Since minimum light transmission by the colour is in the region of 650 $m\mu$ it can be measured photometrically with the use of a red filter. The procedure consists in measuring up to 15 c.c. of the sugar solution (containing not more than 0.4 gm. fructose) into a 100 c.c. volumetric flask, adding water to bring the volume to 15 c.c. if necessary, then adding 5 c.c. of Folin-Denis reagent. After 4 min., 10 c.c. of 20 per cent. trisodium phosphate solution are added, the flask is placed in boiling water for 10 min., made up to volume with water, and the coloured solution compared against water in a 1 cm. cell in the photometer. Colour values are expressed as log 100/per cent. light transmission. Calibration curves are pre-

pared by treating known amounts of fructose and glucose (separately) in the same way, and plotting weight of sugar against colour value. The total amount of fructose and glucose in the solution is determined by the Lane and Eynon or Munson and Walker method. From these data—the colour value and total sugar content of the fructose-glucose solution and the calibration curves—the proportions of each sugar present can be calculated.

A. A. D. C.

Use of Decolorizing Carbon in Determination of Sugars in Plant Materials. A. BEVENUE (*Analyt. Chem.*, 1949, 21, 586–587). The percentage of recovery of glucose, fructose and cane-sugar from water solutions after treatment with different types of decolorizing carbons have been determined. Animal charcoals did not adsorb any measurable quantity of any of the sugars, except cane-sugar at the lowest concentration studied (0.05 per cent.), but with the other carbons the recovery of cane-sugar varied between 43 and 87 per cent. Within useful limits of carbon and sugar concentrations (up to 500 mgm. carbon per 100 c.c.; from 0.1 to 1 per cent. sugar) 10 of the 12 carbons studied did not absorb fructose or glucose, so that in sugar analysis it is advisable to hydrolyse before treating with carbon, and thereby avoid adsorption of cane-sugar. Sugar solutions treated with neutral lead acetate and disodium phosphate prior to the carbon treatment behaved in the same way as the water solutions.

A. A. D. C.

Determination of Pentosans in Cellulose. P. H. TEUNISSEN (*Analyt. Chem.*, 1949, 21, 620–622). The author describes a simplification of TAPPI standard T 450 m 44 method. Instead of distilling 300 c.c. while keeping the volume in the distillation flask constant by addition of 12 per cent. hydrochloric acid, only 180 c.c. of distillate are collected and no addition of acid is made during distillation. In these circumstances less hydroxymethylfurfuraldehyde comes over than in the standard method, and the correction of –1 per cent. necessary in the TAPPI formula for calculating pentosan content in cellulose is reduced to –0.6. The author has found that the 180 c.c. distillate contains all the furfuraldehyde when no acid is added during distillation, although if acid is added as in the standard method the specified 300 c.c. must be collected. This he ascribes to the increase in acid concentration and temperature in the distilling flask in the short method. Continued distillation gives in both cases a small amount of oxidizable materials, but no additional furfuraldehyde. Pentosan determinations on a number of celluloses made by the long distillation-bromide-bromate (TAPPI) method, the short distillation-bromide-bromate method, and

the short distillation-barbituric acid method have given results which show good agreement between the three methods.

A. A. D. C.

Displacement Chromatography on Synthetic Ion-Exchange Resins. I. Separation of Organic Bases and Amino Acids Using Kation-Exchange Resins. S. M. PARTRIDGE and R. G. WESTALL (*Biochem. J.*, 1949, **44**, 418-428). Application of the principle of displacement chromatography to the separation of bases and ampholytes on columns packed with the synthetic kation-exchange resin *Zeo-Karb. 215* (Permutit Co., Ltd., London) is described. Briefly, the procedure (as applied to bases) is to run the solution to be separated on to a column, and follow it with a solution of a base that is more strongly adsorbed than any in the mixture. Once flowing equilibrium has been established, the less strongly adsorbed bases are successively displaced by those having a stronger affinity for the ion-exchanger, and the components of the mixture pass down the column in a series of discrete bands, each containing those components of the mixture which have a similar basicity. In an analogous way a mixture of amino acids may be resolved into fractions each containing ampholytes of similar iso-electric point. Since the bands are not generally visible, a means of following the separations is needed. In this work the separations were followed by observing changes in the composition of the effluent solutions by means of (1) continuous measurement of electrical conductivity; (2) continuous measurement of p_H ; (3) titration of successive small fractions; and (4) qualitative analysis of successive fractions by means of the filter paper chromatogram. Data for the adsorption of various bases and amino acids on *Zeo-Karb. 215* are given in the form of retention isotherms (curves in which the amount of substance adsorbed *per* gm. of adsorbent is plotted against the concentration of the solution). Two components of a mixture of ampholytes could be separated only if their iso-electric points differed by more than 0.5-1.0 p_H unit. Over the range of p_H 1-7 the sulphonic acid residues in the resin were the only reactants with bases, but at higher p_H values the phenolic residues were also reactive. The phenolic reaction is slow to reach equilibrium, so that resins containing residual phenolic groups are unsuitable for the separation of bases stronger than ammonia. Fine grinding of the resin was essential for the production of sharp bands; resin graded between 40 and 60 mesh/in. wire sieves was satisfactory, when used with a rate of progression of the solution down the column of 10-15 cm. *per* hr. Equations have been derived permitting the calculation of the proportion of the column occupied by a component, the width of the boundaries (zones of overlap

between bands), and the expected yields of pure components in separation experiments.

A. A. D. C.

Simple Electrical Meter for Determining the Moisture Content of Grain. H. E. RASMUSSEN and J. A. ANDERSON (*Canad. J. Res.*, 1949, **27F**, 249-252). Several forms of electrically operated meters are now available for determining the moisture content of grain, but most of these involve complex circuits and the use of accurate recording instruments. One of the authors has devised a relatively simple type known as the G.R.L. meter, which should be manufactured and marketed more cheaply. This meter utilizes a sample of grain weighing 190 gm., which is dropped into a cell having two opposite sides of aluminium and thus serving as a condenser. The moisture in the grain increases the capacity of this condenser and this is compensated by reducing the capacity of a second variable condenser by the rotation of a dial until oscillation begins in a triode tube and an electron ray indicator. This is indicated by a change in the shadow angle and the position on the dial is read on an arbitrary scale. The instrument was calibrated by tests made on 159 samples of Canadian wheat of which the moisture was determined in a vacuum oven at 100° C. Dial readings are converted to percentage moisture by reference to a chart, and a small correction for the temperature of the sample is then applied. With samples ranging in moisture content from 10 to 17 *per cent.* the standard error of the results obtained was found to be ± 0.36 .

T. J. W.

Study of the Water Content of Single Grains of Wheat. T. A. OXLEY (*Cereal Chem.*, 1948, **25**, 111-127). Apparatus and technique are described whereby the water contents of single grains of wheat are determined in lots of 40 grains at time. The whole grains are placed separately in shallow conical depressions in a brass ring, the temperature of which is raised by an alternating current induced between an internal iron core and an external exciting coil. The temperature of the ring is maintained at 120-125° C. (248-257° F.) by adjustment of the voltage applied to the exciting coil, and all but the exciting coil is enclosed in a vacuum desiccator containing calcium chloride. Drying is for 48 hr., since it has been found by test that these conditions give the same value for water content as the standard method of drying the coarsely crushed wheat for 4 to 4½ hr. in a ventilated oven at 115° C. (239° F.). Weighing is done on an air-damped, automatically-loaded microbalance reading to 0.01 mgrm., enabling a weighing to be completed within 30-40 sec., in which time the grain water-content does not alter. The standard deviation

of each set of 40 results was used to measure the range in water content, and it has been found that wheat stored in small containers, of the order of 1 litre or less, has a standard deviation ranging from 0.13 to 0.41 *per cent.* of water. A distinction is made between a range of water contents due to grains being in equilibrium with different humidities (the "unsteady state"), and a range due to physical differences in the grains giving differences in water content when all are in equilibrium with the same relative humidity (the "equilibrium state"). The range of water content is temporarily increased by exposure of wheat to very dry or to damp atmospheres which lead to rapid drying or damping of the grains, which shows that the grains differ in the rate at which they are able to exchange water vapour with the atmosphere. In one sample examined, a soft white Scandinavian variety "Als," rapid drying produced a skew distribution of water content. This is evidence of skew distribution among the grains of a character which affects the rate at which they exchange water vapour with the atmosphere. There are indications that the same condition exists in some other varieties. Wheats of mixed origin were generally found to have a wider range of water contents in the equilibrium state than wheat from a single crop. In a single study of a soft red wheat, Squarehead Masters, ripening ears were found in general to dry out first at the apex and last at the base, although a few grains near the base dried as quickly as the apical grains. The range of water content in a single dead-ripe ear was large (standard deviation 0.74), but this was probably partly an unsteady state due to incomplete drying out.

A. A. D. C.

Thiamine and Riboflavin Content of Manitoba Barley of the 1946 Crop. A. D. ROBINSON, L. E. LYND and B. J. MILES (*Canad. J. Res.* 1949, 27F, 241-248). The amounts of thiamine and riboflavin have been determined in 46 samples of four pure varieties of barley grown on 10 different soil zones in Manitoba. The results expressed in microgram. *per* gm. ranged from 3.3 to 4.9 for thiamine and from 0.9 to 1.9 for riboflavin. There is evidence of some varietal difference in the thiamine content but none in the amount of riboflavin. No relation was shown between the quantity of these vitamins present and the nature of the soil on which the barley was grown. There is some correlation between the protein and thiamine contents and also between the amounts of ash and riboflavin, but the values are too small to enable the vitamin contents to be deduced from the other constituents. T. J. W.

Collaborative Analysis of Barley for Thiamine and Riboflavin. E. Y. SPENCER,

A. D. ROBINSON, L. W. McELROY and J. KASTELIC (*Canad. J. Res.*, 1949, 27F, 194-198). Since discrepant results are often obtained in the assay of corresponding samples of cereal for vitamin content in different laboratories, the authors have carried out analyses of samples of barley, wheat, and oats by identical procedures in three co-operating laboratories. Thiamine was assayed by the thiochrome method and riboflavin by the fluorometric procedure, the values obtained being submitted to statistical analysis. Whilst improvement resulted from the standardization of methods in the different laboratories, small but consistent and significant errors still remained. Calculations showed that differences of 10 to 12 *per cent.* in mean assay values were necessary before the thiamine or riboflavin contents could be considered significantly different. T. J. W.

Unfermentable Reducing Substances in Molasses. L. SATTLER and F. W. ZERBAN (*Industr. Engng. Chem.*, 1949, 41, 1401-1406). About 10 *per cent.* of the reducing power of the unfermentable substances of cane molasses is due to volatile constituents. To obtain information on the nature of these constituents, a 75 *per cent.* fructose solution was refluxed for 16 hr., cooled, diluted and completely fermented with bakers' yeast. After removing the yeast, and evaporating *in vacuo*, the thick syrup was dehydrated by azeotropic distillation with ethanol and benzene. Sugar anhydrides were precipitated from the residue by taking up in hot methanol and pouring into dry ether. The methanol-ether solution was evaporated in *vacuo* and the presence of hydroxymethylfurfural, acetoin, acetol, methylglyoxal and levulinic and formic acids established by qualitative tests on the residue. These substances are thermal or fermentative decomposition products of sugars. Some of Euler's speculations on melanoidin formation (this *Journ.*, 1944, 147) were substantiated, but there was no evidence that methylglyoxal was the only sugar degradation product responsible for caramel and melanoidin formation. Several types of caramel and melanoidin, in varying degrees of polymerization, probably exist, depending on the nature of the sugar, the impurities present in a given product, temperature, p_H and concentration. Some unfermentable melanoidins yield fermentable sugars on hydrolysis and must therefore be derived directly from sugars. C. R.

II.—FERMENTATION

Pathway of Adaptive Fermentation of Galactose by Yeast. J. F. WILKINSON (*Biochem. J.*, 1949, 44, 460-467). This investigation was made with an extract of a galactose-adapted Dutch top yeast, prepared under

anaerobic conditions in order to conserve its full activity. It has been shown that such a yeast produces adaptively an enzyme, which the author proposes to call galactokinase, and that this enzyme catalyses the phosphorylation of galactose by transference of the terminal phosphate group from adenosine triphosphate to form α -galactose-1-phosphate. The enzyme is activated by magnesium ions and cysteine. It is unlikely that galactose-6-phosphate is an intermediate in this reaction because no yeast extract yet investigated can ferment this ester. Moreover, the author's work shows that the enzyme system which forms galactose-1-phosphate from galactose is unable to attack galactose-6-phosphate, that there is no initial formation of an acid-stable ester during the phosphorylation of galactose by the extract, and that the extract is unable to phosphorylate tagatose, whereas yeast hexokinase will phosphorylate fructose and mannose in addition to glucose. A portion of the phosphate esterified was found to be present as an acid-stable phosphoric ester with a soluble barium salt. The supposition is made that, if this ester proves to be glucose-6-phosphate, then the transformation of galactose-1-phosphate to fructose-1,6-phosphate (which has been shown elsewhere to be formed in the course of galactose fermentation) probably takes place *via* glucose-6-phosphate or an ester in equilibrium with glucose-6-phosphate in the system described. There is no evidence of the accumulation of galactose-1-phosphate during the pre-adaptive period.

A. A. D. C.

Fermentation of Trehalose by Yeasts and its Taxonomic Implications. R. J. BOUTHILLET, N. E. NEILSON, E. M. MRAK and H. J. PHAFF (*J. gen. Microbiol.*, 1949, 3, 282-289). In the classification of yeasts, it is customary to use an extract of bakers' yeast as basal medium for the determination of sugar fermentations. Investigating a strain of *Candida tropicalis*, an apparent fermentation was observed of both maltose and lactose. Since according to the Kluyver-Dekker fermentation laws, a yeast will not ferment both maltose and lactose, the anomalous behaviour was investigated further. The yeast extract used was shown to contain trehalose which was fermented by *C. tropicalis* by means of an adaptive enzyme system. The use of an extract of bakers' yeast may therefore lead to unreliable results and it is considered desirable to use yeast autolysate in preference to the extract. Autolysate is prepared by incubating equal weights of bakers' yeast and water at 55° C. for 72 hr.; for use it is diluted with nine parts of water. All the trehalose is fermented during autolysis. As an alternative, an extract of brewers' yeast is often satisfactory, since the latter contains no trehalose.

With yeast autolysate as basal medium, the fermentation of trehalose has been tested for 133 strains of yeasts from 20 genera and 73 species. The fermentation of this disaccharide appears to have some diagnostic value, particularly in the genus *Candida*. Several other genera contain strains capable of fermenting trehalose, a case of particular interest being the hybrid *S. carlsbergensis* X *S. cerevisiae*.

A. E. W.

Assimilation of Glutamic Acid by Yeast.

E. S. TAYLOR (*J. gen. Microbiol.*, 1949, 3, 211-223). By Gale's amino-acid decarboxylase technique the amino acids arginine, glutamic acid, histidine, lysine, ornithine and tyrosine were shown to be present, in the free condition, within the cells of the yeasts Carlsberg No. 237 and 174 ("Yeast Foam" and Dutch top yeast). Although present when growth took place in the absence of the amino acids, an increase within the cells was noted when the latter were included in the media. Glutamic acid metabolism was investigated in greater detail and its uptake found to be dependent on an energy source which can be provided by glucose fermentation. The rate of uptake and total uptake of glutamic acid was decreased by the presence in the medium of ammonium salts and certain amino acids. If the cells were suspended in salt solutions without amino acids, the glutamic acid content was unaffected unless glucose was added, when a decrease was noted. The concentration remained constant when ammonia was present, indicating either the synthesis of glutamic acid or the preferential utilization of ammonia.

A. E. W.

Yeast Growth Factors. L. ATKIN (*Wallerstein Lab. Commun.*, 1949, 12, 141-151). The history of the study of yeast growth factors is traced from the time of Wildier's bios (1901) to the present day. It is now recognized that bios comprises at least six individual growth factors. Few yeasts require all six factors, but many require at least one, two, three, or more. Bios I or inositol is hexahydroxycyclohexane; it occurs in malt sprouts, tea dust and brewers' yeast and in the latter may be associated with lipids. The insecticide "gammexane" possesses a chemical structure similar to inositol and its properties may depend upon an antagonistic mechanism. Bios IIA or pantothenic acid consists of one molecule of β -alanine linked by a peptide bond to one molecule of β -dimethyl- α - γ -dihydroxybutyric acid. β -alanine functions equally well as a growth factor, but does not occur free in nature. Biotin or Bios IIB is a dicyclic compound and is extremely potent; 1×10^{-4} microgram. in 10 ml. causes a measurable increase in yeast growth. These three factors comprise bios as originally conceived and a combination

of the three is satisfactory for the growth of most yeasts. Some yeasts require additional factors, *viz.*, vitamin B1 or aneurin (thiamin) and vitamin B6 or pyridoxin. Aneurin molecules comprise two portions, a substituted thiazole and a pyrimidine moiety; yeast is capable of uniting these two fragments and also capable of storing fairly large quantities of aneurin; for the most part aneurin exists in a combined form as co-carboxylase. Vitamin B6 (pyridoxin) occurs widely in nature in at least three different forms and functions in an amino-acid-decarboxylase system. Sometimes an additional factor (nicotinic acid) is required by yeasts, particularly lactose fermenting strains. Brewers' yeast contains (in microgram. *per* gram.) the various factors in the following quantities:—

inositol	..	..	3,000–5,000
pantothenic acid	..	..	80–150
biotin	..	..	2–2.5
aneurin	..	..	100–150
pyridoxine	..	..	20–40
nicotinic acid	..	..	400–600

A. E. W.

Growth and Fermentation Factors for Different Brewery Yeasts. L. ATKIN, P. P. GRAY, W. MOSES and M. FEINSTEIN (*Wallerstein Lab. Commun.*, 1949, 12, 153–170). The influences of bios factors on the growth of various yeasts and on fermentation by them, were first studied separately and finally investigation was made into the effect on combined growth and fermentation such as takes place in brewery practice. Using a synthetic medium from which individual growth factors could be omitted and estimating yeast growth photo-electrically, it was found possible to group yeasts into five "bios-types" which required the addition of (a) biotin only; (b) biotin and pantothenate; (c) biotin and inositol; (d) biotin, pantothenate and inositol; (e) biotin, pantothenate, inositol and thiamin (aneurin) or pyridoxin. Lager yeasts belong to the first four types and ale (top-fermentation) yeasts to the last type. The bios-types remained constant after long storage of slants or daily serial transfers, although the ability to synthesize the factors was sometimes slightly weakened. The bios-typing was of value in assessing the continued purity of lager yeasts in actual practice. Fermentation tests were carried out with a heavy yeast inoculum using a synthetic medium of different composition from that used above. It was possible to reproduce a rate of fermentation similar to that obtained with beer wort and the carbohydrates of this medium were glucose (4 parts) and maltose (1 part). Although aneurin and pyridoxin were incorporated in the final medium, the bios-factors were found to have little effect on fermentation, as distinct

from their profound effect upon yeast growth noted above.

Finally, a buffered synthetic medium containing 7 *per cent.* of carbohydrate was inoculated with the yeasts at pitching rates comparable with those of brewery practice. It was found that the addition of factors was necessary for attenuation rates comparable with beer wort results to be realized. This is probably a reflection of the increased yeast growth required to maintain attenuation at its correct rate. Of the various bios-factors, inositol is of dominant importance under these conditions and the omission of inositol had an effect as great as the omission of all the bios-factors. The inositol requirement for attenuation appears to be unrelated to whether or not this factor is required for growth of the yeast used, and it is possible that this fact may be due to the inability of yeasts to store this factor. Brewery wort contains quantities of inositol some 20 times more than the limiting value required; it is therefore unlikely that deficiencies of this factor will be encountered in practice, but the possible occurrence of inositol anti-metabolites should be borne in mind.

A. E. W.

Trehalose from Yeast. L. C. STEWART, N. K. RICHTMYER and C. S. HUDSON (*J. Amer. chem. Soc.*, 1949, 71, 2277). Crystalline trehalose was obtained by a modified Myrbäck and Örtenblad method (this *Journ.*, 1937, 237; 1938, 240). Preliminary treatment involved deproteinization with zinc sulphate and baryta, followed by de-ionization with exchange resins. Depending on the history and age of the yeast, yields up to 12 and 20 gram. *per* kilogram were obtained from brewers' yeast and bakers' yeasts respectively. The content in yeast can be preserved by storage of crumbled yeast under 95 *per cent.* ethanol.

C. R.

Bacteriological Control in the Brewery. P. CHEVALIER (*Brasserie*, 1949, 3, 119–122). The author condemns the application to beer of methods used in the bacteriological examination of water, and especially the tests for coliform bacteria, as being unnecessarily involved and serving no useful purpose. The occurrence of coliform bacteria in beer has been noted only in abnormal circumstances, when unsuitable materials had to be used and the beer was of extremely low gravity. No quantitative significance could be attached to the number present (often very high), for either they were derived from some non-faecal pocket of infection in the brewing materials, or a few introduced at some point from the coolers onwards had proliferated enormously by the time the beer came to be tested. Furthermore, no case is known of such beers, infected with coliform and other undesirable types of bacteria, having caused

trouble. For bacteriological work in the brewery, beer itself is the medium of most general use, contained in small bottles with airtight closures and pasteurized twice at 65–70° C. (149–158° F.), but it is not very sensitive and is slow to give results. Beer sterilized at 100° C. (212° F.) in tubes or flasks closed with cotton wool is more sensitive, because it has lost its carbon dioxide and some of its alcohol, but its sensitivity is increased further by the addition of yeast water. Increased sensitivity is, however, gained at the expense of specificity. A medium which allows "sarcinae," lactobacilli and wild yeasts to develop, while restricting mycoderma and acetic bacteria, consists of a mixture of 2 parts of beer to 1 part of yeast water, sterilized in full, 15 c.c. flasks which are closed with waxed corks. Inoculations are incubated for 4 days at 25° C. (77° F.). Wort can be tested in this way after fermenting it with a pure yeast if the presence of beer disease organisms is sought, or, if information on the wort organisms is wanted, the wort can be examined microscopically direct and after 24 and 48 hr. incubation at 25° C. The presence of pockets of infection of bacteria of coliform or putrefactive types can be detected by inoculation into yeast water. Brewing water is best tested by incubation in the beer-yeast water medium and in beer only. Plate counts on beer gelatin or agar afford a measure of filtration efficiency, but a nephelometer reading would be simpler and quicker. Exposure of petri dishes containing wort gelatin or agar will give some indication of the organisms present in the air at the point of exposure, but the results cannot be taken as quantitative. All the results of bacteriological examination should be interpreted in the light of local conditions and it is unsafe to generalize. Isolated results can prove to be misleading; it is the consistently kept record of results over a period that is informative.

A. A. D. C.

Wort Bacteria. E. HELM (*Reprint, Schweiz. Brau-Runds., Special Congress No., 1949, 4 pp.*). The term Wort Bacteria covers the group of organisms about one micron in length and generally to be found in samples of wort, fermenting wort, yeast, and water. Whilst it is not possible in routine microscopical examinations to differentiate between the various genera and species, the wort bacteria (being Gram-negative) may readily be distinguished from other rods, cocci or bacteria. In 1948 Olsen and Poulsen found that the wort bacteria comprised chiefly members of the genera *Aerobacter*, *Escherichia*, *Flavobacterium*, *Pseudomonas* and *Achromobacter*.

The author has examined 615 samples from different breweries; aliquots of these samples, cultivated in yeast-water for three days, afforded

growth of wort bacteria in 254 samples. The samples were also tested for acid and gas in lactose-peptone medium, positive results being obtained in 232 cases. In 52 of the samples, positive results were obtained for acid and gas when no growth was obtained in yeast-water. A positive result from lactose-peptone solution whilst indicating the genera *Aerobacter* and *Escherichia* must be confirmed by other tests and from the 232 acid and gas producing samples, only 95 were identified as members of these two genera. Twenty of the samples were shown to contain *E. coli* of the indole positive type and six samples contained the indole negative type of the same species. It is the latter which is responsible for a phenol-like taste in beer, and this peculiarity has been reproduced experimentally by inoculating sterile wort with the indole negative strain of *E. coli* and after a suitable time adding a pure culture of yeast. The phenol taste was detectable after four days of fermentation. It appears to be necessary for the wort to receive a period of incubation with the organism prior to pitching with yeast for the full development of the off-flavour. The other members of the *coli-aerogenes* group appear to be capable of causing celery-like or mushroom-like flavours in beer.

The author concludes by advocating high standards of personal cleanliness, the starting of fermentation as soon as possible after the finish of boiling, and a routine examination for members of the *coli-aerogenes* group of organisms by means of lactose-peptone medium.

A. E. W.

Brewing Bacteriology. VII Common but Harmless Brewery Bacteria. J. L. SHIMWELL (*Wallerstein Lab. Commun., 1949, 12, 187–193*). Beer spoilage bacteria often grow poorly on solid media, and when searching for them plates may easily be encumbered by rapidly growing and commonly occurring harmless species. It is therefore necessary to be able to discriminate between the two types, particularly as morphological resemblances are not uncommon. The family *Micrococcaceae* includes the genera *Micrococcus*, *Gaffkya* and *Sarcina*, of which only *Gaffkya* is unlikely to be encountered. The two other genera may be confused with the beer-spoilage streptococci, but mistakes can only arise from morphological and Gram-staining similarities, as their growth requirements are distinctly different. The aerobic members of the genus *Micrococcus* which are most usually encountered in brewery work may be distinguished by their catalase-positive nature. As beer-spoilage types the micrococci can probably be disregarded, but since some are hop-tolerant the possibility must not be ignored that acclimatization may produce beer-disease

strains. The spore-forming bacteria (*Bacillaceae*) are seldom encountered in beer; indeed, if they were, pasteurization would be of little value. They are, however, often found in many brewing materials. The genus *Clostridium* is characterized by the possession of spores larger than the rods, carried either centrally or terminally, and the genus includes many species of industrial importance in other fermentations. Aerobic spore-formers of the genus *Bacillus* are often encountered, the commonest species being *B. subtilis*, *B. cereus*, *B. mycoides* and *B. megatherium*. Many other species have been recorded and it is suspected that much synonymy might exist in the group. For example it has been recorded that *B. mycoides* widely recognized as a distinct species may dissociate into a variety of *B. cereus*. Likewise the culture of Brown's *Bacterium X* was found to be a strain of *B. cereus* and related to *Bacterium C* (Chapman), which was a different strain of the same species (cf. this *Journ.*, 1937, 339). The aerobic spore-formers are readily recognized as such and are easily distinguishable from, e.g., the lactobacilli.

A. E. W.

Examination by Partition Paper Chromatography of the Nitrogen Metabolism of Bacteria. H. PROOM and A. J. WOIRWOOD (*J. gen. Microbiol.*, 1949, 3, 319-327). Three hundred strains of bacteria have been grown in a medium containing casein-hydrolysate as nitrogen source. After growth, the filtrates have been examined by a paper chromatographic technique devised by Woiwood in which ten filtrates were run simultaneously on one paper. The changes in amino acid composition of the medium may reflect either a group or species significance. Bacteria with simple growth requirements, indicated by their ability to utilize ammonia, do not affect the chromatogram in their initial growth phase. If the chromatogram is affected, the first amino acid to be metabolized is serine. In the case of Gram-positive bacteria, the aspartic acid spot is the first to be eliminated and the basic amino group is not affected; the reverse occurs with Gram-negative bacteria. Some bacteria appear to effect a synthesis of polypeptides, indicated by the appearance of a new ninhydrin-positive spot on the paper, and the appearance of a given type of spot may also have group significance. By disintegrating bacteria and examining the cell contents it has been shown that many strains are capable of storing amino acids, but there is little evidence of polypeptide storage.

A. E. W.

Recent Developments in Methods of Testing Antiseptics. G. F. REDDISH (*Amer. quart. J. Pharm.*, 1949, 127-148). The method prescribed by the U.S. Food and Drug Administration for

testing liquid antiseptics utilizes 2 per cent. phenol for comparison, a medium of specified composition and a highly resistant strain of *Staphylococcus aureus* as test organism. The test has been found satisfactory, but it has been criticized on the grounds that it does not simulate practical conditions. More recently proposed tests involving tissue or *in vivo* methods are generally unreliable except for research purposes, nor has it been found possible to obtain a reliable method for determining the toxicity of an antiseptic, although the use of leucocytes or of test animals has been suggested. Mercurial germicides offer some difficulty in test owing to their high bacteriostatic powers, whereby carryover of the germicide to subculture media prevents multiplication of viable organisms. This difficulty has been largely overcome by the use of thioglycollate as the subculture medium. Fungicides may be tested by the agar cup plate or other technique. The complete evaluation of an antiseptic depends on (a) bactericidal activity; (b) bacteriostatic activity; (c) influence of organic matter; (d) speed of action; (e) penetrability; (f) toxicity.

A. E. W.

III.—BEER

Simple Method of Sparge Water Treatment. G. W. SENG. (*Brewers' Digest*, 1949, No. 5, 43, 48). The process of sparging, whilst an economic necessity, yields a dilute extract which tends to have an adverse effect upon the final wort, but this may be minimized by previous treatment of the sparge water. Experimental brews showed that such treatment produced a beer with a smoother and more pleasant hop flavour, but difficulty was experienced in dissolving the gypsum used for treatment. This was overcome by a method described earlier by Smith and Hagen in which a slurry of gypsum and water was pumped into the pipe-line conveying the sparge water from the tank to the sparge arms. Owing to the abrasive action of the suspended gypsum, the impeller and drive shaft of the pump were rapidly damaged. To avoid this the author substituted top pressure of carbon dioxide instead of a pump for transferring the slurry. The apparatus used was a closed vertical cylinder fitted with a funnel for introducing the water and salts, a stirrer near the base operated by an electric motor above the cylinder, and a connection to a supply of compressed carbon dioxide. The calculated amount of salts was stirred with water and poured into the cylinder which was then filled with water. Whilst being rapidly stirred the required volume of slurry was then gradually forced into the stream of sparge water with which it became intimately mixed.

T. J. W.

Role of Proteins and Tannin in Wort Boiling. H. LUERS (*Brewers' Digest*, 1949, No. 5, 39-42). The author summarizes the results obtained by himself and other investigators on the various factors influencing the precipitation of proteins by tannin and the action of these substances during the boiling of wort. Wort contains proteins and tannins derived from the malt, and the addition of hops contributes a larger amount of tannin. An increase in the amount of protein precipitates larger quantities of tannin on boiling, but no proportionality exists between the reactants at different concentrations, in practice an excess of tannin always remains. Among the various factors causing an increased adsorption of tannin by proteins are: a rise in temperature, partial oxidation of the tannin by exposure to air, reduction of the p_H value of the wort to 5.0 or lower, an increase in the time allowed for reaction, and the presence of tannin whilst the higher proteins are beginning to coagulate by heat. Whilst tannin is readily adsorbed by the heat-coagulable proteins it also combines with albumoses and peptones of relatively low molecular weight, forming compounds which are soluble, but unstable, and are liable to produce haze or cold turbidity in the finished beer. Tannin contributes a harsh, astringent flavour to beer which accentuates the bitter taste of the other constituents present, and in some breweries the practice of scalding the hops in boiling water before adding them to the wort has been adopted. This procedure removes about 40 per cent. of the tannin present, but has little effect on the other substances present and produces beers having a finer and milder flavour, although the copper break occurs later than when untreated hops are used. The author expresses the opinion that hop tannin is undesirable in worts and beers and refers to a method recommended by Windisch in which the hops are ground and extracted with cold water. The aqueous infusion is then treated with an adsorbent material such as activated carbon which removes practically all the flavour, odour and colour, whilst the soluble proteins and tannin remain. The liquid is then added to the portion of the mash (in the decoction system) which is to be boiled. By this procedure, the copper wort is freed from all coagulable proteins and hop tannin, and it is then boiled with the extracted hops. The resulting beer is free from abnormal colour, aroma and taste and shows a greatly reduced tendency to the formation of cold turbidity.

T. J. W.

Maintenance and Improvement of Beer Quality. E. CHABOT (*Fermentatio*, 1949, No. 1, 1-9). If a brewer is not satisfied with the quality of his beer he is unwise to try and brew an imitation of some other brewer's more

popular product. He would be better advised to give scrupulous attention to every detail of his brewing, from the choice of barley to the time the beer leaves the brewery, and so produce a beer having its own distinctive character. Beer may be of three kinds; excellent, good except for one or two minor defects, or poor. If the beer is excellent the brewer's task is to keep it so, by studying every detail of his process so that he knows the value of every factor and is unaware of none. He should know his barleys, the way they are malted, and the kind of analytical results to expect from his malts. His water treatment should be regularly checked by analysis and no detail of mashing, boiling and cooling left to chance. He should choose his hops with care, being guided by analysis in regard to their content of resins and, later, how they are keeping. The choice and care of his yeast is of great importance, and a pure culture should be maintained from which replacements may be worked up when needed. He avoids access of air to his beer at all points after fermentation, and relies on a planned, routine system of biological control to warn him of incipient infection. The brewer whose beer is in the second class should first try the effect of a few simple changes such as raising or lowering the hop rate or the gravity, storing the beer longer or giving it slightly more carbon dioxide. If these are of no avail he can then investigate his process point by point, taking care in his investigations to change only one detail of procedure at a time. It is seldom in this case that any fundamental change is required, but rather some small, easily overlooked fault that needs rectifying. The improvement of a poor beer is easiest of all, because the faults of material, plant, or process are generally obvious. As before, however, every detail should be checked from the earliest stages onwards, special attention being given in this case to replacing out-of-date methods and plant. A poor beer is often the result of allowing plant to deteriorate or become obsolescent. Finally, the brewer should not consider his task is complete when the beer leaves the brewery, but should use all the influence he can to ensure that it gets properly handled and served by the licensee.

A. A. D. C.

Fobbing Beer ; its Nature, Causes and Remedies. G. B. BEATTIE (*Bottling*, July, 1949, 20-38). Fobbing is defined as copious foam formation due to the release of gases in solution at a rate greater than normal. The fobbing of beers resembles that of soft drinks, but that of the latter is simpler and more under control. The presence of air is of value in the early stages of production and when the finished beer is dispensed, but in all intermediate stages it may be a nuisance. Agitation of beer at any

stage either before or after carbonation tends to eliminate some of the carbon dioxide and induce fobbing later, and for this reason all pipes in a bottling store should be of large diameter with smooth inner surfaces and free from sharp bends or obstructions. Although it is essential to keep beer properly carbonated up to the point of bottling, this increases the difficulty of quiet filling. Such filling reduces foaming, thus preventing elimination of air from the bottle neck, and so leads to the formation of a poor head when the beer is dispensed. Agitation and air are probably the chief fob-producing factors, but the formation of foam is also increased by a rise in temperature before or during carbonation, excessive chilling of the beer which causes denaturing of the proteins, over carbonation, colloidal particles in the beer due to incomplete filtration, the presence of dirt or dried alkali in badly washed bottles, and filling into bottles which are warm. To reduce fobbing, uniform top pressure should be maintained during carbonation, and means should be provided for taking off the air liberated from the beer during this operation. In relation to this, one investigator has shown that the passage of soft drinks through certain pulp filter sheets causes a noticeable reduction in the air-content of the beverage. All joints, gaskets, *etc.*, should be tight, the bottling rate should not be increased, and the beer should run into the bottles with minimum turbulence. There appear to be many contradictory and conflicting factors concerned in the formation of fob, but a small amount of air in the bottle appears to be essential for the production of a satisfactory head when the beer is dispensed, although larger quantities have an adverse effect upon the character and duration of the foam.

T. J. W.

Spectrophotometric Measurement of Colour in Worts and Beers. G. VAN ROEY (*Bull. Assoc. anc. Etud., Louvain*, 1949, **45**, 16-22). When a number of worts, beers and beer colouring materials of varying depths of colour were optically analysed in a spectrophotometer, it was found that the series of curves obtained by plotting percentage light transmission against wavelength were of the same form. Maximum absorption was in every case at about 400 m μ and minimum at about 615 m μ , although there was a slight, regular shift of the minimum towards a longer wave length with increase in depth of colour. When optical density (logarithm of the reciprocal of the transmission) was plotted against colour as measured with 0.1 N iodine, the relationship was found to be linear for worts, beers, *etc.*, and for the iodine at 615 m μ , but at 400 m μ there was a departure from linearity which was greater for the iodine than for the beers. The substitution of Hellige colour glasses for the iodine as a means of colour

measurement gave better results at 400 m μ , and it became possible to express colour in terms of optical density at this wavelength (which, being the point of maximum absorption, is the most sensitive point at which to take optical readings). A small correction is necessary if the liquid has more than a certain depth of colour, owing to the increasing value of the optical density at 615 m μ . Tests made on worts and beers of varying degrees of haziness have shown that preliminary clarification is not essential when using the spectrophotometer. The results were not significantly different from those obtained by means of the Hellige glasses after the liquids had been clarified by filtration with kieselguhr.

A. A. D. C.

IV.—PLANT, MACHINERY AND BY-PRODUCTS

Physics of Bottling. J. BRAEMS (*Bull. Assoc. anc. Etud., Louvain*, 1949, **45**, 23-42). The degree to which a beer maintains its quality unimpaired during bottling depends primarily on two things: the extent to which it retains its initial carbon dioxide content, and the extent to which atmospheric oxygen is excluded. Carbon dioxide in a well-conditioned beer in tank is in a very finely dispersed state, and this dispersion has to become coarser before gas can form bubbles and be evolved. The factors governing dispersion are saturation, pressure, temperature, time and certain hydrodynamic forces brought into play by movement of the beer. Air can be introduced either by injection by the jet of beer entering the bottle, or by slow diffusion from the atmosphere above the beer in the tank, filling machine, or closed bottle. The amount injected depends on the speed, size and type of movement of the jet; the faster the speed, the greater the surface area, and the less smooth the movement, the greater the quantity of air entrained. Diffusion increases with increase in temperature or pressure of the gas above the beer. Bottling efficiency, as far as quality of the beer is concerned, is governed by these facts. Rise in temperature or fall in pressure between tank and filling machine should be avoided. If some rise in temperature is unavoidable it is an advantage to cool the beer in tank to allow for the rise, and the bottles entering the machine should be as cold as possible. The relative positions of tank and filling machine must be studied and steps taken to offset the drop in pressure due to the diminishing head of beer in the tank. The pipes connecting the two should be kept short, be as wide as possible, and have no abrupt changes in size or direction. The filler-head should be of medium size, and it should be easy to wash the inside of it. The pressurizing of the bottles should be complete by the time the beer is

admitted, and filling should be as slow as is consistent with the bottle being just full by the time the jet is cut off. Speed of filling is best controlled either by a venturi-type constriction in the feed pipe, by using a narrower air-return pipe, or by a combination of both these means. The filler nozzles should be designed to give a smooth flow, should reach well down into the bottle so that the jet is short, and should preferably be of the non-emptying type. The author deals in some detail with the reasons for these recommendations, and the bearing which each has on the fundamental aims of conserving the carbon dioxide equilibrium of the beer and reducing its contact with air to a minimum.

A. A. D. C.

Plastics in the Brewing Industry. F. F. JARAY (*J. Incorp. Brewers' Guild*, 1949, **35**, 257-265). The two principal classes of plastic materials are those described as thermoplastic and thermosetting varieties; both can be softened by heat, moulded into any required shape and then allowed to harden on cooling. The difference between the two is that whilst those of the thermoplastic type can be again softened on heating, those of the thermosetting type retain their hardness at all temperatures. The thermosetting varieties of which Lithcote, Prodor-glas and Heresite are examples, have been used successfully as linings for steel vessels during the last 15 years, but they are attacked by alkalis and if damaged cannot be repaired satisfactorily. Polythene and polyvinyl chloride are examples of the thermoplastic group and have been widely used for pipe lines. They are unaffected by acids and alkalis, may be drilled, cut and turned like metals, and can be welded by means of a current of hot air. They may be coated on to steel and concrete with some difficulty and one variety known as Nutravac has been used for fermentation vessels. This material is very tough and any damaged area may be readily repaired by applying and vulcanizing a patch. Owing to the expansion and contraction of wood, Nutravac is applied with difficulty to this material. This plastic is being applied in the form of removable linings for metal drums, and although in this form it is unsuitable for use in wooden casks, it should be satisfactory for those constructed of steel or other metal. The plastics of the furane resin class have been used as bonding for tiled floors, giving excellent service for years. T. J. W.

V.—BIOCHEMISTRY

Crystalline α -Amylase from Malt. S. SCHWIMMER and A. K. BALLS (*J. biol. Chem.*, 1948, **176**, 465-466; through *Amer. Brewer*, April, 1949, 110). An enzyme solution was purified by a method utilizing the heat stability of α -amylase, its stability in the presence of

calcium, and the adsorption of the crude enzyme from 40 per cent. alcoholic solution on starch. Addition of ammonium sulphate at p_H 5.9-6.0 at 30° C. to the purified enzyme solution gave a crystalline protein, the activity of which, per unit of protein nitrogen, was 67 times that of the original malt extract.

C. R.

Influence of Salts on Amylase Activities of Malt. V. L. ERLICH and G. M. BURKERT (*Cereal Chem.*, 1949, **26**, 239-257). A study was made of the effect of salts, particularly those found in industrial waters, on the extraction of amylases from malt. 10 gm. finely-ground malt was extracted with 200 ml. distilled water or salt solution at 20, 30, and 40° C. for times varying from 10 min. to 24 hr. Starch hydrolyses and determination of diastatic power were conducted according to the methods outlined in *Cereal Laboratory Methods* (1941) and dextrinizing activity was determined by a modification of the method of Sandstedt, *et. al.* (this *Journ.*, 1940, 52).

The α -amylase activity of malt stored for periods up to 115 days, varied more than D.P. or β -amylase activity, but the variations could not be correlated with duration of storage. Compared with water, extracts made with low salt concentrations gave variable, but increased, α activity for all malts, rising to an optimum with concentrations of about 0.1 N. The highest optimum was obtained with sodium bicarbonate. The lowest salt concentrations gave higher values when extraction was carried out at 30 rather than 20° C., but this difference almost disappeared at the optimum concentration for extraction. Concentrations of salts higher than the optimum depress the α -activity of extracts. By selection of salts, maximum α -activities were obtained, which were not increased by raising the extraction temperature to 40° C., nor by increasing the time of extraction. The maximum α -activity was characteristic for each particular malt, but the ratio of maximum activity to the activity of water extracts varied from malt to malt. The conditions of optimum extraction approximate to those prevailing in breweries.

Extraction with salts gave only small increases in β -activity. Higher concentrations of some salts, *e.g.*, calcium and magnesium chloride and sodium thiocyanate, reduced the activity, especially for extracts made at 30° C. The increased D.P. at low salt concentrations was due to the increased α -amylase activity. No marked activation of α -amylase occurred when salts in low concentrations were added to water extracts, but some ions caused slight stimulation of β -activity. Both α - and β -activities of water extracts were depressed by addition of higher salt concentrations, the effect being due to inhibition of the enzymes by ions. C. R.

Hydrolysis of Waxy Maize Starch by Pancreatic Amylase. F. M. MINDELL, A. L. AGNEW and M. L. CALDWELL (*J. Amer. chem. Soc.*, 1949, 71, 1779-1781). Hydrolyses of branched-chain whole starches were carried out with highly purified, maltase-free pancreatic amylase. The waxy maize starches used gave no evidence of the presence of linear components. Hydrolyses were carried out at 40° C. under conditions protecting the enzyme from inactivation (this *Journ.*, 1948, 341). Results obtained under comparable conditions and using the same enzyme concentration indicated that the waxy starches hydrolysed more slowly than ordinary unfractionated maize starch and much more so than the linear fraction from maize starch. Over wide limits, the extent of hydrolysis of the waxy starches depended on enzyme concentration, but unlike β -amylase of malt, the extents of hydrolysis attained with different concentrations did not tend to reach a common limit (this *Journ.*, 1946, 319). Waxy maize starch hydrolysed rapidly to products of relatively low molecular weight and high reducing power (*cf.* this *Journ.*, 1949, 255). When 20 per cent. of the glucosidic linkages were broken, 62 per cent. of the total reducing power and 87 per cent. by weight of the products were accountable as dextrans polymerized to an average of 6.8 glucose units. Attack of the enzyme on the branched chain seemed to be random at first. Subsequently, hydrolysis slowed down, due largely to the replacement of substrate by products hydrolysed only slowly and for which amylase had low affinity. When 98 per cent. theoretical maltose was reached, dextrin with an average of 4.4 glucose units accounted for 36 per cent. by weight of the products. These dextrans were of somewhat higher molecular weight than the equivalent residual dextrans from unfractionated starch or linear fractions (this *Journ.*, 1949, 255). Maltose and glucose were formed from waxy starches by the maltase-free amylase. Maltose was present in concentrations of 2-5 per cent. from the earliest stages of hydrolysis, whereas glucose appeared later and in smaller concentrations. C. R.

Isolation of Isomaltose from Enzymic Hydrolysates of Starch. E. M. MONTGOMERY, F. B. WEAKLEY and G. E. HILBERT (*J. Amer. chem. Soc.*, 1949, 71, 1682-1687). Two procedures were used to isolate 6- $[\alpha$ -D-glucopyranosyl]-D-glucose (isomaltose) from exhaustive taka-amylase digests of waxy maize starch. In one, fermentable sugars were removed from the digests with bakers' yeast, the yeast-free filtrate being clarified and passed through ion-exchange columns. More enzyme was then added and hydrolysis continued four days longer. Treatment was repeated until no change in rotation and reducing power was observed. After removal of protein with basic lead acetate

and further passage through exchange columns, the solution was concentrated *in vacuo*, the resultant syrup taken up in methanol and impurities were precipitated by treatment with ethanol and ether and cooling to 0° C. for 48 hr. The supernatant was concentrated to a colourless syrup, dried *in vacuo* over P_2O_5 and dissolved in 1 part of butanol plus 2 parts of methanol. On slow concentration *in vacuo* at 30° C., granular, non-crystalline, crude isomaltose separated. The sugar was identified by conversion into three interconvertible crystalline derivatives. The structure of the sugar as 6- $[\alpha$ -D-glucopyranosyl]-D-glucose was established by means of an octa-acetyl derivative which was identical with Wolfram's octaacetyl-6- $[\alpha$ -D-glucopyranosyl]- β -D-glucose (this *Journ.*, 1949, 254).

The second preparation avoided the possibility of introduction of isomaltose by yeast. 200 grm. starch was made into a 5 per cent. paste at 82° C., cooled to 38° C. and digested with 10 grm. taka-amylase for 24 hr. in presence of toluene. After Seitz filtration, digestion was continued until reducing power and rotation were constant (96 hr.). The digest was clarified and ions removed by passage through exchange columns. All sugars were adsorbed at p_H 7-8 on carbon-celite columns, glucose being eluted by washing with water. Isomaltose was then eluted with 0.5 per cent. aqueous phenol. On concentration and drying, a syrup was obtained, which on acetylation and crystallization from ethanol at 25° C. gave pure octaacetyl-6- $[\alpha$ -D-glucopyranosyl]- β -D-glucose. Deacetylation of the product was carried out catalytically with barium methylate at 0° C., barium was removed with sulphuric acid and the filtrate concentrated *in vacuo*. The resultant syrup, taken up in ethanol, crystallized in long prisms during several days. C. R.

Maltase Activity of Mould Brans. R. L. GATES and E. KNEEN (*Cereal Chem.*, 1949, 26, 228-239). The evaluation of glucose in presence of maltose is a necessary preliminary to maltose assay. A roughly quantitative maltase assay method was developed using a commercial dehydrated yeast, having a pronounced induction period before maltose was fermented and during which glucose was selectively fermented. For the period between 30-90 min., CO_2 production was closely related to glucose content over a range of sugar concentrations. Fermentation was measured manometrically in mercury manometers. Enzyme extracts for examination were prepared by extracting 10 grm. bran for 1 hr. at 30° C. with 100 ml. water. For each determination of maltase activity, three manometer cups were necessary, each containing 15 ml. of a solution of mineral salts, aneurin and pyridoxin at p_H 5.8. To one cup was added 600 mgrm. glucose and to each of the others

600 mgrm. maltose. Aliquots of enzyme solution were added to each cup to make up a total volume of 25 ml. One cup containing maltose was heated 3 min. on the boiling water bath to serve as a blank. Each cup was then rapidly brought to and maintained 1 hr. at 50° C., then heated 3 min. in boiling water and cooled. After bringing to 30° C., 10 ml. of a suspension containing 1.5 gm. dried yeast ("Maca") was added, and the cups fixed to the manometers. Pressures were read after 30 and 90 min. The blank determination was taken from those obtained with active enzyme solutions. Glucose production (mgrm.) in the maltose cup was given by:

$$\left(\frac{\text{Corrected maltose reading}}{\text{Corrected glucose reading}} \right) \times 600$$

Provided aliquots of bran solutions were chosen so as to give not more than 40 per cent. maltose conversion, the glucose produced from 600 mgrm. maltose was proportional to maltase quantity.

Brans were compared by *maltase unit value*, defined as mgrm. maltose converted to glucose by the action of 1 gm. of mould bran in 1 hr. at 50° C. Brans varied widely in maltase activity. There was little relation between maltase and amylase activity, except within a group of brans prepared from closely related moulds, e.g., the *Aspergillus oryzae* or *Rhizopus-Mucor* groups. Barley malt had no measurable maltase activity. The procedure is of value in studies of the concentration and separation of bran carbohydrates.

C. R.

Preparation of Amylose by Thymol Precipitation. S. LAKSHMANAN, J. SRI RAM and K. V. GIRI (*Current Sci.*, 1948, 17, 366). Amylose of comparable purity to that given by Hassid's methanol method (this *Journ.*, 1943, 263) was obtained by a modification of Haworth's thymol method. Sweet potato starch was dispersed in water and poured into water at 80° C. to make a 1 per cent. solution. After stirring frequently at 80° C. during a period of 1½ hr., the solution was cooled and centrifuged. Sodium chloride to 0.1 per cent. and thymol to saturation were added immediately to the supernatant. The precipitated amylose was centrifuged off after 24 hr., and washed with thymol-water, absolute alcohol and ether.

C. R.

Oxidation of D-Galactose and Cellulose with Nitric Acid, Nitrous Acid and Nitrogen Oxides. W. W. PIGMAN, B. L. BROWNING, W. H. MCPHERSON, C. R. CALKINS and R. L. LEAF, jun. (*J. Amer. chem. Soc.*, 1949, 71, 2200-2204). The specific oxidation of primary alcohol groups in carbohydrates by nitrogen dioxide prompted investigations on the oxidation of galactose to mucic acid. Moisture influenced the rate of oxidation, as judged by a CO₂-evolution technique, being markedly retarded by the

presence of phosphorus pentoxide. The yield of mucic acid from galactose by evaporation at 100° C. with nitric acid is usually 70-80 per cent. Reproducible yields of 88-90 per cent. were, however, obtained by the following method. To 10 gm. galactose and 1 gm. sodium nitrite mixed dry in a bath at 0-3° C. was added with stirring 30 ml. of ice-cold nitric acid (70 per cent.). The mixture was kept at 0-3° C. for 4 hr., then allowed to stand at room temperature for 20 hr. The solution was diluted with 20 ml. of saturated mucic acid solution, the crystals filtered off and washed with saturated mucic acid. High yields were also obtained if, instead of using sodium nitrite, the nitric acid was treated (a) by saturation with nitric oxide; (b) at 0-3° C. with nitrogen dioxide; (c) with copper turnings; (d) with barium or silver nitrite. About 10 per cent. of galactose, probably converted mainly to galacturonic acid, was not recoverable as mucic acid. Studies of the changes in rotation of galactose during oxidation with nitric acid saturated with nitric oxide and analysis of the oxidized solution at the stage of maximum negative rotation indicated that the oxidation proceeded: D-galactose → D-galactonic acid (or γ-lactone) → L-galacturonic acid (or γ-lactone) → γ-lactone of mucic acid → mucic acid. On oxidation of galactose with fresh, concentrated nitric acid at 0-3° C., no appreciable change in rotation took place in 200 min. Subsequently, oxidation did occur, possibly due to the slow formation of reaction products derived from the acid. In the presence of sodium nitrite, nitric acid induced a rapid change in rotation from a positive to a negative value. This reaction was inhibited by urea, which is known to remove nitrous acid. When the nitric acid was previously saturated with nitric oxide, oxidation commenced immediately and proceeded at about the same rate as when sodium nitrite was added. The oxidation of cellulose by nitric acid containing sodium nitrite yields fibrous polyuronides. Whilst such treatment is less hazardous than the use of the highly toxic nitrogen peroxide usually employed, yields under the conditions used were lower.

C. R.

Molecular Properties of Water-soluble Polysaccharides from Western Larch. G. L. BORGIN (*J. Amer. chem. Soc.*, 1949, 71, 2247-2248). The water-soluble extracts made at 30° C. from the heartwood and sapwood of a 30-year-old butt-log of Western larch were respectively 8.4 and 0.9 per cent. of the wood. Sedimentation velocity runs showed that the heart wood extract contained one component, whereas the sapwood extract contained two, present in the proportion 3:2, the higher molecular weight component, probably identical with that in the heartwood, being present in greater amount.

C. R.

JOURNAL OF THE INSTITUTE OF BREWING

MONTHLY REVIEW

BREWING INDUSTRY RESEARCH FOUNDATION

DR. A. H. COOK, F.R.I.C., formerly Assistant Professor in Organic Chemistry at Imperial College and Reader in the University of London, having been appointed Assistant Director of Research to the Brewing Industry Research Foundation entered upon his duties as from 1st October, 1949. The position of Controller of Applied Research has been accepted by Mr. B. M. Brown, B.Sc., F.R.I.C., and others who have accepted invitations to join Sir Ian Heilbron's staff at Lyttel Hall, Surrey, are Dr. J. L. Shimwell, F.R.I.C., and Dr. I. A. Preece, F.R.I.C., F.R.S.E. The members of the Institute Research staff who are now working at Birmingham University will in due course also be transferred to the new establishment at Lyttel Hall.

* * * *

EUROPEAN BREWERY CONVENTION

THE first Council Meeting of the E.B.C. to be held in Great Britain took place at the office of the Brewers' Society in London, on 2nd November last. M. Phillippe Kreiss (President) was in the chair,—and in addition to the Secretary, Dr. F. Mendlik, the following representatives were present:—J. de Clerck, J. Segard (*Belgium*); H. Fogh, N. Steenberg (*Denmark*); B. M. Brown, N. B. Smiley (*Great Britain*); B. D. Hartong, J. A. Emmens (*Netherlands*); A. Ringnes (*Norway*); E. Olson, H. Lundin (*Sweden*); and H. Huerlimann, K. Schoellhorn (*Switzerland*).

The Council examined the results of the Lucerne Congress and some time was devoted to discussing the proportion of time which should be given at the Congresses to practical as distinct from purely scientific subjects. It was decided to leave the proportion very much as at present with one

strictly scientific subject and one technological subject, with the proviso that the latter must be treated scientifically.

It was agreed that the next Congress should be held at Brighton in the week commencing 28th May, 1951, and that the special subjects to be considered will be:—

- (1) Primary fermentation in the brewery with particular attention to the flocculation of yeast.
- (2) Aspects of the economic functioning of the Brewery:
 - (a) Losses of material in brewing, Losses of material in racking and bottling;
 - (b) Mechanization and new technique in the brewery and bottling plant.

It was agreed that papers to be read at the Congresses should in the first place be submitted to the organization which is the member of the Convention representing the author's country, *i.e.* in the case of an English author his paper will be submitted to the Institute of Brewing which will decide if the paper is suitable for presentation to the Congress. There was some discussion as to the desirability of publishing papers in full before the Congress, and it was decided that for the next Congress only abstracts will be published before the Congress and a condensed version of the full paper should be read at the Congress; full publication would follow if the author so wished.

Reports were received from the Analysis and Barley Committees. It was agreed to adopt the latter's recommendation that barley growing trials of one well-known variety of barley should be carried out in as many countries as were able to co-operate and the resulting samples submitted to malt and brewing trials as well as to analysis. For the first trial it was decided to adopt Kenia barley.

During their visit Members of the Council of

the Convention and their wives were guests of the Institute at the Opera (Covent Garden) and at a Dinner held in their honour at the Savoy Hotel. They were also entertained to luncheon at the Park Royal Brewery by the Directors of Arthur Guinness, Son & Co., Ltd., and to cocktail parties given by Mr. M. V. Courage (President of the Institute of Brewing) and Mrs. Courage, and by Mr. A. P. Lennox-Boyd, M.P., respectively, the latter being held at the House of Commons.

* * * *

LODGING OF CEREALS

MUCH is heard of this topic, but it is seldom that the layman has the opportunity of reading a reasoned survey of the problems involved. However, an interesting article by H. I. Moore (*Agriculture*, 1949, 56, 314-316), reviews observations made over the past 15 years for the causes of lodging in 500 fields, representing an area of 7,350 acres devoted to wheat, barley and oats. The causes have been classified under 10 different headings. The most frequent (31 *per cent.* of all cases) seems to lie in the choice of varieties having, *e.g.*, straw either too long or too weak. High soil fertility was responsible in 7 *per cent.* of cases, and might have been obviated by growing potatoes or beet beforehand. Greater care in rotation would probably have helped in those cases (5 *per cent.*) where disease was responsible for the trouble. Deficiencies of phosphate appeared responsible in some cases, and the use of nitrogenous fertilizers too early in the season in others. Excessive seed rates were blamed in 7 *per cent.* of cases; winter proudness may also cause trouble. It is significant that nearly 80 *per cent.* of the recorded cases seem to have been due to controllable causes; only 21 *per cent.* were attributed to such weather causes as wind, flooding and storm, and here stiffer strawed varieties might have minimized the trouble. The user of malting barley will doubtless wish to be assured that, in selecting this desirable stiffness, the malting quality of the grain itself will not be sacrificed.

* * * *

TENTH ANNIVERSARY OF V.E.B.O.

BEFORE the last war Belgium grew only some 6 *per cent.* of the malting barley and only half of the hops which were required for the extensive brewing industry flourishing

there, and, in 1938, a few people not only realized this, but proceeded to organize the necessary changes to maintain the industry even if the imminent threat of war should develop. Thus, organized by Prof. Isebaert, V.E.B.O. came into existence—an organization for encouraging the cultivation of improved varieties of barley and hops.

On 28th, 29th and 30th October this year, the tenth anniversary was celebrated in connection with the annual barley and hops exhibition and the presentation of prizes for the winning samples. The awards are based on a points system, which is the same as that in use by the corresponding organizations in France (SECOBRAH) and in Holland (Na Co Brouw). All three of these organizations work in close collaboration, particularly in co-ordinating the methods and results of the varietal trials conducted by the three countries.

In the anniversary celebrations on 29th October, after a morning session devoted to the barley and hops exhibition and to addresses dealing with barley, the awards were presented to the winners by His Excellency Monsieur Orban, the Minister of Agriculture, at a luncheon attended by representatives of the Government, of the malting and brewing industries, and of barley research in Denmark, France, Great Britain, Holland, Norway and Sweden.

During the Congress addresses were given on:

- (1) *The Influence of Barley Husks on the Quality of Beer*, by Prof. L. Isebaert.
- (2) *Survey of Barley Selection in Western Europe*, by Dr. H. Van Veldhuizen.
- (3) *A Consideration of the Collaboration in the Belgian, French and Dutch Barley Variety Trials*, by Dr. P. Bergal.
- (4) *A Review of the Ten Years of V.E.B.O.*, by Prof. L. Isebaert.

* * * *

CEREAL SEED

A HANDBOOK under this title has recently been issued by the Institute of Corn and Agricultural Merchants, Ltd. (5, Copthall Chambers, London, E.C.2) and The National Association of Corn and Agricultural Merchants, which contains much information of general interest, though it is intended

primarily for seedsmen and seed growers. To quote from the opening of Chapter I:

"The task of the cereal seed merchant is to make arrangements for growing and supplying to his customers the cereal seed needed, at the right time, and of the best variety for its purpose."

The implications of this requirement are then discussed. Following old custom, some merchants still breed their own varieties, but most rely on specialist breeders for a nucleus stock, and trials are carried out to test suitability for particular conditions; important help is given by N.I.A.B., as in preparing recommended lists of varieties after test. The choice of ground for growing cereals for seed holds a number of pitfalls, since contamination with other cereals must be avoided. In general, merchants prefer grain which has "sweated" in rick, since with such grain fewer weeds are likely to be present; germinative capacity and dormancy are also important factors. It is clear that combine harvesting presents new problems here, as it does also to the maltster, and it is not surprising that drying problems are receiving increased attention. An important modern advance is the use of organic mercurial compounds for seed dressing, leading to a high measure of control of many seed-borne diseases, including stripe and covered smut of barley.

Notes on the choice of varieties include a discussion of such topics as the type and fertility of land, and other factors of importance in this connection are the intended use of the grain and the way it is to be harvested. Some pertinent remarks about barley appear under the heading of *Seed Rates*, the importance of uniformity of sample being stressed, this involving seeding at such a rate that tillering is discouraged. Growing, harvesting and threshing are dealt with in useful outline in relation to seed-grain production, where every care must be taken to avoid introduction of foreign seeds and damage to germinative capacity—matters which are of equal interest to maltsters; it is noted that barley is particularly sensitive to dampness in stack. Reference is also made to the development of cereal inspection schemes, designed to ensure authenticity of variety and freedom from disease and noxious weeds, whilst annotated tables of cereal varieties are also given; thus, 18 barley varieties are listed, including three (Bere, Pioneer and Prefect)

under the heading of *Winter Barleys*. It is of interest that Kenia and related varieties are given as of poor malting quality. The booklet concludes with notes on legumes and linseed, and a chapter devoted to the *Seeds Act and Regulations*.

This is a useful publication from which brewing students will gain much that will be of benefit to them and even more experienced men will read it to their profit. Published in July, 1949, it is obtainable from the address quoted above (by post 2s. 9d.).

* * * *

BARLEY IMPROVEMENT

REFERENCE has been made on a previous occasion (this *Journ.*, 1949, 194) to the work of the *Mid-West Barley Improvement Association* of U.S.A., and the continuing educational efforts of the Association are worthy of further note, providing as they do a wealth of literature relating to such topics as the choice of varieties for growth in the areas concerned, fertilizer treatment, and the production of threshed samples acceptable to maltsters. As a means of procuring crop uniformity, arrangements have been made for three counties—one each in Minnesota, North Dakota and South Dakota—to restrict their barley growing to one variety each (Kindred "L" in the first two, Odessa in the third), growers also undertaking to observe a number of rules in regard to seed dressing, fertilizer treatment and use of clean ground, and harvesting and subsequent treatment. In turn, co-operation is promised from managers of grain elevators, merchants, brewers, agricultural colleges and county agents. Thus, a full advisory service is provided throughout, it should be possible to keep the samples free from admixture with others, and a market should be assured.

The influence and value of the advisory service is evidenced by the type of literature which is being distributed. Thus, yield comparisons are provided of a wide range of barley varieties grown at numerous stations (J. W. Lambert) and report is given of fertilizer trials carried out in 1948 (R. L. Willits), though there seems little novel in the results of these trials; however, mention of the possibility that excessive nitrogenous fertilizers could possibly increase lodging may serve as a useful reminder to

some growers. Emphasis is laid on proper threshing, carried out so as to avoid damage to grain. In an article *Malting Barley is more than Variety*, S. L. Vogel (reprinted from *The Gleaner*, May, 1949) states that only half the growers of malting barley addressed by his remarks offered barley acceptable to maltsters—the barley submitted by the rest contained more than 5 per cent. of damaged corns. Such an opening naturally leads to the presentation of suggestions as to how this may be avoided on future occasions by careful and periodic attention to the adjustment and running of the thresher. Again, B. D. Leith writes on *Suggestions on Growing and Harvesting Malting Barley*; some of the points he makes may not be directly applicable to British conditions, though there are harvesting hints which strike a familiar note. Thus, when cutting with a binder the crop must be fully ripe with yellow straw and hard grain; then—"with the combine, wait three to five days longer." In this country a somewhat longer period of waiting has often been advocated in recent years, but clearly the idea is the same. There are obviously problems in common on both sides of the Atlantic, and the results accruing from the distribution of this mass of literature will be awaited with interest.

* * * *

GRAIN PESTS

AN article entitled *Infestation of Farm-Stored Grain*, and "intended only as a general guide on the infestation problem," appears in the October, 1949, issue of *Agriculture* (56, 316-321), and contains much information of general interest. Brief descriptions of the pests are given and the type of damage caused is described, including the hollowed or holed grains associated with weevil, the matted development caused by the spinning activities of moth caterpillars, and the dustiness and minty smell associated with mites. There is also the well-known cumulative danger of heating, even in comparatively dry grain, where disturbance only aggravates the trouble by spreading the insects responsible. Principles governing general precautionary measures will be well-known to those concerned with grain storage in connection with malting, including as they do the observance of cleanliness and the use of structures in a good state of repair, the

dangers of heat, the possibility of infecting one stock from another, and the desirability of keeping sacks away from walls. For treatment of infected material, heat may be used, but only with care since it is necessary not to impair the desired characters of grain—such as germinative capacity. Fumigants can be used on bags, and a variety of insecticides have been used on infected grain, but DDT and benzene hexachloride cannot be employed for foodstuffs as their toxicity to man and animals is not known. Several types of treatment are available for bins and the like, but are unlikely to be effective without preliminary thorough emptying and cleaning; such cleaning alone may be sufficient, or it may be followed by special insecticidal treatment, using a dust or spray containing DDT or benzene hexachloride—the dust may be the more suitable in buildings of loose construction, whilst with hard non-absorbent surfaces, oil sprays may be used. Powders of suitable potency for spraying on to absorbent or white-washed surfaces are available, and will retain their effectiveness for weeks so long as they do not become covered by dust or removed by frequent brushing.

* * * *

PARTITION CHROMATOGRAPHY

READERS of the Abstracts section in this *Journal* will have noticed numerous references to chromatography in its various forms during recent years, and will probably have been impressed by the elegance of the technique involved and its very real suitability for application to a wide variety of biochemical problems; it needs little imagination to think of many problems in brewing science which might be helped—in some cases have already been helped—towards a solution by the application of chromatography. Adopted first for the separation of amino acids (*cf.* this *Journ.*, 1947, 171), partition chromatography has since proved extremely valuable also for the separation of sugars, following the work of S. M. Partridge (this *Journ.*, 1949, 247). It is of particular interest, therefore, that the Proceedings of a Biochemical Society Symposium held on 30th October, 1948, have now been published (*Partition Chromatography*, edited by R. T. Williams and R. L. M. Synge. Cambridge: University Press, 1949, price 6s.), providing

a review of numerous types of problem which have been investigated by this technique. As is remarked by Synge in considering the applicability of partition chromatography, it has already been used for effecting separations in most of those groups of low-molecular organic compounds which interest biochemists, though it is not confined to organic compounds but can also be used with microgram quantities of certain inorganic materials. The use of filter paper, though a very old device, has recently come very much to the fore in frontal analysis, and some very delightful techniques have been worked out. Thus, Partridge in his chapter dealing with carbohydrates mentions some of the reagents which can be used for "developing" the sugar spots on the paper chromatograms, it being possible to use reactions characteristic of pentoses, or reactions distinguishing ketoses from other sugars; quantitative working is also possible.

Silica gel was first used for separating and estimating amino acids, and has proved particularly useful in the case of mono-amino acids derived by hydrolysis of peptides and proteins. Paper chromatography has since been used with considerable success. Studies of protein structures and of amino acid and protein metabolism are discussed by F. Sanger and C. E. Dent, respectively. In a detailed investigation of tissue fluids, many known amino acids have been detected and a number of "unknowns" present have been provisionally identified. Thus, α -aminobutyric acid, γ -aminobutyric acid, methionine sulphoxide and methyl histidine have been found. There are certain difficulties which must be taken into account: two-dimensional working is much more specific than one-dimensional and identifications should be confined to materials stable to acid hydrolysis, whilst mixed running of the "unknown" both with its pure supposed counterpart and with other amino acids allows of the highest precision. It is worth noting that the two aminobutyric acids mentioned above have also been obtained from bacterial sources by E. Work, who has also studied the changes in medium composition during bacterial growth.

Numerous other types of compound can be dealt with. E. C. Bate-Smith discusses certain pigments and related compounds, whilst S. R. Elsden describes application to organic acids, purines and pyrimidines. Other

important chapters are contributed by A. A. Levi, on the use of stationary phase materials other than water, and by A. J. P. Martin, on theoretical aspects of the subject. In many cases the main papers are supplemented by further information arising in the course of discussion; an extensive bibliography is given. As is pointed out by E. Baldwin in his introduction to the Symposium, quantities of a fraction of a mgrm. and upwards of material can generally be used for examination if suitable conditions of working are chosen, and the potential value of such a method in investigating some of the lesser known constituents of brewing materials (and clearly in the possible isolation of constituents at present unknown) will be evident. The publication of the proceedings of this Symposium represents a considerable help to workers in this field.

* * * *

THE SECTIONS

LONDON SECTION

A JOINT meeting with the London and South of England Section of the Incorporated Brewers' Guild was held at the Horse Shoe Hotel, London, W.1, on 10th October, Mr. C. L. Downing, Chairman of the Guild Section presiding. Mr. G. Osgood, M.A. (Cantab.), F.R.I.C., M.I.Mech.E., who lectured on *Sterile Filling*, described the various items of plant required to secure the bottling of sterile beer, illustrating his lecture with diagrams to show details of construction and manipulation. After removing all organisms from the beer by filtration, it is run into bottles which have been sterilized by moist sulphur dioxide. The actual bottling must be done on a specially designed filler which can be sterilized and maintained in a sterile condition throughout the day's bottling. The routine of sterilizing the filter and bottling machine was described and the need emphasized for adequate screening of the conveyor lines from the bottle sterilizer through the filler to the moment when the bottle is closed with a sterile crown cork or screw stopper. A bottle washer incorporating a sterilizing filter for the rinse liquor was now being developed and was expected to give sterile bottles and so avoid the need for sterilization by sulphur dioxide. The discussion which followed the reading of the paper served to emphasize the claim that

when the prescribed precautions were observed, beer with a very long bottle life could be obtained more economically by sterile filling than by pasteurization.

A joint meeting with the Food Group of the Society of Chemical Industry, was held on 24th October. Mr. B. M. Brown, who presided, was supported by Mr. A. L. Bacharach, Chairman of the Food Group. The speaker was Dr. H. Lundin, Professor of Food Chemistry at the Royal Institute of Technology, Stockholm, who lectured on *The Microbial Synthesis of Fats*. Dr. Lundin introduced his lecture with a review of the work which had been done in various countries on fat formation by micro-organisms and on their utilization as sources of fat and protein foodstuffs and as a contribution to the vitamin requirements of the human dietary.

In recent years an extensive study had been made in Sweden directed chiefly to fat production potentialities, and Dr. Lundin described the process which had been evolved for the cultivation of *Rhodotorula gracilis* from carbohydrate and mineral salts. The yeast prepared by this process contained over 40 per cent. of fat on dry matter. The results of analyses of the yeast from a dietary point of view were discussed, these including examination of the component fatty acids in the fat and of the essential amino acids in the yeast protein as well as assay of the B-vitamins and of provitamin A. Preparations of *R. gracilis* would provide a useful adjunct to a diet in which carbohydrate predominated.

In the discussion which followed, various speakers described the lines on which study of fat-formation in micro-organisms was being undertaken in this country. The possibility of economically extracting the fat from the yeast manufactured by the Swedish process was discounted, but it was claimed that the yeast as a whole would be a valuable addition to the normal diet in countries where animal protein and fat were scarce, and it was generally agreed that the process could be employed most profitably in tropical countries where cheap fermentable carbohydrate was available.

At the meeting on 14th November, Mr. B. M. Brown, Chairman of the Section presided, and a paper entitled *English*

Barleys was read by Mr. H. Leslie Thompson, who commented on the extremely dry weather conditions which had prevailed throughout the year. The better samples of barley owed their maturity to good root development rather than to the adventitious rain which fell shortly before harvest. The eastern counties and the south and west produced a reasonable quantity of first-class spring barley and there were some fine winter barleys, but the crop in many of the larger barley-growing areas was rather disappointing. In many districts the Danish varieties were widely grown. This year they gave excellent yields and some of the crop was of reasonably good malting quality, but the lecturer considered that such barleys could not be relied on to give consistently good results under the varying weather conditions which were experienced from year to year. The number of combine harvesters was increasing rapidly, and when judiciously used they were giving excellent results; but the problem of drying and storage still required urgent attention. As so many lecturers had done in recent years, Mr. Thompson again emphasized that sweating was a stage of the malting process which required the skilled attention of the maltster, and could not be entrusted to farm driers.

In the discussion which followed, speakers agreed that the Danish varieties were giving quite good malt this year but expressed grave misgivings about a general displacement of the well-tried English varieties, especially as trade conditions were such that brewers would become more critical about the quality of malting barley.

* * * *

MIDLAND COUNTIES SECTION

At a meeting held at the Midland Hotel, Birmingham, on 20th October—Mr. B. Wood in the Chair—papers on the subject of *Cask Timbers* were read, respectively, by Mr. E. Doyle and Mr. W. J. Bull.

Mr. Doyle described the various species of timber available for cask manufacture, and gave some account of the geographical distribution of timber-growing regions. Prior to 1939, brewers would only accept staves of cleft oak, but since that time various types of timber—cleft sawn and in many cases sawn lumber—have provided useful substitute materials. During 1939 and to

the end of the war, only British and American timbers were available, but there have since been importations from Persia, Poland, Jugo-Slavia, France and Rumania, as well as from America. Qualities from Poland and France have been mixed, but good samples have been seen from Rumania and Jugo-Slavia. The Persian timber is beautifully quartered, but sometimes contains interior shakes. Mr. Doyle's paper was illustrated by photographs and maps, and he concluded by discussing the probable range of future supplies from the various sources.

Mr. Bull said that, up to 1800, English oak was chiefly used for beer casks, but Russian oak was introduced about that time; early conditions of supply led to some wastage, but producers soon introduced a specification acceptable to consumers which minimized this. With a cessation of Russian supplies about 1924, Poland became the chief exporter, providing a timber which was highly satisfactory both to the cooper and to the brewer, though results were slightly inferior in quality and finish to those from Russian timber. Conditions during and after the war again led to changes in the sources of supply (*cf.* above). The timbers now being imported are not, from a variety of reasons, entirely satisfactory. The American timber should be lined, and is inclined to blister and spalch; breakages of French timber would be reduced if the proper quality and cleft were supplied, whilst lining is also desirable here as it is with the Yugo-Slavian. Persian timber is very suitable without lining, but a high proportion received during the past two years has been shivered, a difficulty it should be possible to overcome if practical supervision were provided throughout production.

* * * *

SCOTTISH SECTION

MEMBERS of the Section were the guests of the Scottish Section of the I.B.G. at a meeting held at the North British Hotel, Edinburgh, on 1st November; Mr. J. M. Wilson was in the Chair, and a paper entitled *The Season's Hop Crop* was read by Mr. H. L. A. May, who said that, in the main, the crop had a somewhat greenish appearance, though the seed was mauve or black indicating true ripeness. Possibly, in normal years, inclement or windy weather caused a so-called "ripe" appearance which was, in fact, a weathered

one. This year there had been no such weathering, whilst a further contribution to the greenness may have been an influence of lack of rain on chlorophyll content. This had been a Golding year, probably because the East Kent district, where most of the Southern Counties Golding hops are grown has a heavy soil with deep chalky subsoil, holding moisture better than the gravels of the Weald and Mid-Kent. Hence, in a dry season such as 1949, hops on chalky land with better retention of previously deposited moisture, would be more favoured than those in other areas.

The Hops Marketing Board's estimates of production had just been published, and with one or two exceptions showed a decrease in all districts apart from East Kent, where there was a increase of 16 *per cent.* The Weald was down by over 17 *per cent.* and the Midlands district by just over 9 *per cent.* Fuggles would show better P.V. than last year, and were good hops for all purposes, but the yield would be less in most cases than in 1948. New Varieties showed an improvement; it had been a particularly good year for them, since there had been little trouble from disease.

Home brewers' and export requirements were estimated at 250,000 cwt. compared with 278,000 cwt. for 1948, a decrease of 10 *per cent.* Brewers had this year put in their estimated requirements three months earlier than usual, and so had a lesser period to work on as regards existing trade; secondly, the estimate was made at a time when the trade was at its lowest since 1939. Even so, it appeared from figures published since January, 1949, that the estimated figure of 247,000 cwt. for home consumption was too high, but as published percentages of contracts which will be delivered were only 89 *per cent.*, brewers would only get 220,000 cwt. At a hop rate of 0.9 lb. *per* bulk barrel, estimated requirements of hops from the 1949 crop seem to be only just over 218,000 cwt., which would be covered by the above figure for actual deliveries.

The Section met also at the North British Hotel on 23rd November under the Chairmanship of Mr. L. Fletcher, to hear a paper on *Tests of Germinative Capacity in Barley*, by Miss A. M. MacLeod, B.Sc. (Heriot-Watt College, Edinburgh). The lecturer, describing her own investigations into this subject, said that a 1 *per cent.* aqueous solution of

2, 3, 5-triphenyl tetrazolium chloride had been used as test reagent for germinative capacity of mature malting barley, dormant barley and barley which had been damaged by heating. With mature samples, especially those of high germinative capacity, results obtained by staining with tetrazolium chloride agreed well with those obtained from actual germination tests. The ultimate germinability of dormant samples could be accurately predicted by tetrazolium staining immediately after harvest, when germinative energy varied from 9 *per cent.* to 80 *per cent.* in different samples. With barley which had been damaged by heating, tetrazolium staining proved unreliable; over 90 *per cent.* of the corns might be stained in samples in which there was less than 60 *per cent.* germination.

* * * *

GOLFING SOCIETY

Yorkshire and North-Eastern Section.—Starbeck, 7th October, 1949.

Invitation Cup (Medal)—

Winner: J. Wise, 72 nett.

Runner-up: A. A. Stewart, 74 nett.

Captain's Prizes (Fourball-bogey)—

Winners: A. Maule and J. B. Stewart,
6 up.

Runners-up:

G. I. Semper and B. Rawlings }
P. Branston and R. B. White } 1 up.

Midland Counties and Burton-on-Trent Sections.—Brocton Hall, 9th October, 1949.

Grant Cup Bogey Competition—

Winner: L. Finch, 1 down.

Runners-up: S. Barrington }
M. Armstrong } 2 down.

London Section.—Coombe Wood, 11th October, 1949.

Moritz Cup—

Winner: S. E. Mammatt, 35 points.

Runners-up: T. Wright }
A. C. Clark } 34 points.

Foursomes—

Winners: R. Jupp and J. C. West, 4 up.

MEETING OF THE MIDLAND COUNTIES' SECTION HELD AT THE WHITE
HORSE HOTEL, CONGREVE STREET, BIRMINGHAM, ON THURSDAY,
24th MARCH, 1949

MR. B. WOOD in the Chair.

The following paper was read and discussed:—

GRAIN STORAGE WITH SPECIAL REFERENCE TO BARLEY
STORAGE FOR MALTING

By F. R. JOLLEY, M.Sc.(Tech.), A.M.I.C.E.

Grain storage problems are reviewed and an outline is given of factors concerned in the design of modern silos, the use of concrete, wood and steel being referred to. An improved system of bulk storage in deep bins is described, means being provided for ventilation with either fresh or conditioned air.

Magnitude and Urgency of the Current Problem.—The Chairman of Associated British Maltsters, Ltd., Mr. H. N. Hume, C.B.E., M.C., drew attention in his annual report to the effect of mechanization in agriculture, necessitating the provision of alternative systems of storage, and to the fact that the malting industry had offered to provide such storage so far as malting barley is concerned, subject to certain conditions. It was further pointed out that the new method of harvesting by use of combines involved the acceptance of barley containing a higher proportion of moisture and rubbish in a shorter period of time than by the time-honoured method of storing in the stack or rick and subsequently releasing it as required to pass into consumption.

One of a number of stipulations by way of compensation to the maltster for providing increased storage capacity and plant for sweating barley was that the Government must grant the necessary building licences, supplies of steel and other materials, and labour to carry out the scheme.

It will be appreciated, in view of the foregoing statement, that the problem of grain storage is one of vast and increasing importance to this country; the urgency of the

problem arises partly from the economic conditions resulting from the world war, which have necessitated greatly increased production of native grain, and partly, as already stated, from the modern method of harvesting.

Table I shows the estimated tonnage of wheat and barley harvested in the United Kingdom in recent years.

The acreage under barley in the United Kingdom last season was well over two million, and it is estimated that between one-quarter and one-half the total crop was dealt with by combine harvesters.

Maltsters-for-sale and brewer maltsters have been urged by the Ministry to accept combine harvested barley to their maximum capacity during the harvesting period. Comparatively small progress has been made in constructing additional storage capacity, either for wheat or barley for malting, though some extra drying plant has been made available.

Consideration is being given by the Ministries concerned to the question of licences for the buildings necessary to provide additional storage for native grain, particularly for barley for malting, but as far as the writer is aware, little has been done to

TABLE I
ESTIMATED QUANTITIES IN TONS OF WHEAT AND BARLEY HARVESTED
IN THE UNITED KINGDOM IN RECENT YEARS

Grain.	Average 1935-1939.	1946.	1947.	1948.
Wheat	1,675,000	1,965,000	1,667,000	2,281,000
Barley	780,000	1,935,000	1,619,000	2,010,000

date in the way of actual granting of such licences. Applications made by maltsters, millers and merchants are usually subjected to detailed scrutiny by the regional licensing officers, and even when licences are granted, they are issued subject to approval by the officer concerned of the steel and timber quantities to be used in the construction.

In present circumstances, therefore, the prospects of reasonably prompt authority to proceed depend largely on the production of designs involving the minimum use of constructional material in short supply.

Factors Governing Design of Silos.—Factors governing the design of a silo fall naturally into two classes:—

- (a) Factors concerned with the condition of the grain.
- (b) Factors concerning construction of the silo.

Considering the former, so far as the maltster is concerned, barley must retain both a high germinative capacity and germinative energy, and a great deal of research has been done by many workers to determine the limits of conditions which satisfy these requirements. Spontaneous production of heat in a grain bulk may be prevented by storing at either below about 14 *per cent.* moisture content, or below about 46° F., although prolonged storage of damp grain, even at low temperature, causes the vitality eventually to be slightly impaired. Grain which is to be stored for several months should be brought as near as possible to these limiting conditions unless it is subjected to controlled ventilation. Bearing these facts in mind, the design of a silo should therefore be such as to prevent (a) uncontrolled exposure of grain to humid air, (b) condensation of moisture on surfaces in contact with the grain, and (c) heating of grain by conduction through the bin walls.

Insect infestation is another possible cause of heating of a grain bulk during storage, and it cannot be permanently arrested by merely cooling to a low temperature. In this connection, prevention is better than cure, and a silo should therefore be designed to minimize the possibility of insect attack in fresh grain coming in for storage.

As a further principle, it is hardly necessary to point out that in selecting grain-handling equipment no machinery should be used which is liable to cause physical damage to

the grain; from the maltster's point of view only whole undamaged corns are of any value.

Considering, secondly, factors concerning construction, it is of course necessary in the first place to determine the storage capacity required, and the employment of the site to the best advantage with regard to access both by road and rail, and possibly by water. At the same time, consideration must be given to the suitability of the ground for the loads to be carried, which usually vary between 1½ and 2½ tons *per sq. ft.*; this may become a primary factor in deciding on the suitability or otherwise of a site.

An important factor, which unfortunately is usually left to a late stage, is the consideration of the handling operations and general facilities to be provided within the silo. Thus, in a silo which is primarily for storage, the handling plant can be of comparatively low capacity, whereas in a silo primarily used as a merchant or transit silo, the handling capacity will be correspondingly higher. The importance of this factor will be appreciated when considering the larger export and import silos. For example, one large port silo of 140,000 tons storage capacity, has received and delivered 200,000 tons in a single month. It will be appreciated immediately that the fundamental factor determining design is the handling operations involved, which, in this case, include six intake streams each of 500 tons *per hour* capacity, and 12 streams of similar capacity loading to ship.

It is of paramount importance, when considering design, that the handling operations should be determined before construction is begun. In order to determine the size and number of bins, the handling operations should, of course, be considered in conjunction with the variety and size of consignments to be stored, and the period of warehousing required for the grain. If this procedure is followed, there will be no difficulty in accommodating the machinery and equipment necessary for performing these internal handling operations.

It cannot be stressed too strongly that the efficient operation of a silo is possible only if it is planned and designed in detail before construction is commenced, so that the machinery may be accommodated with the minimum wastage of space. The importance of this point will be fully appreciated when we bear in mind that most architects assess

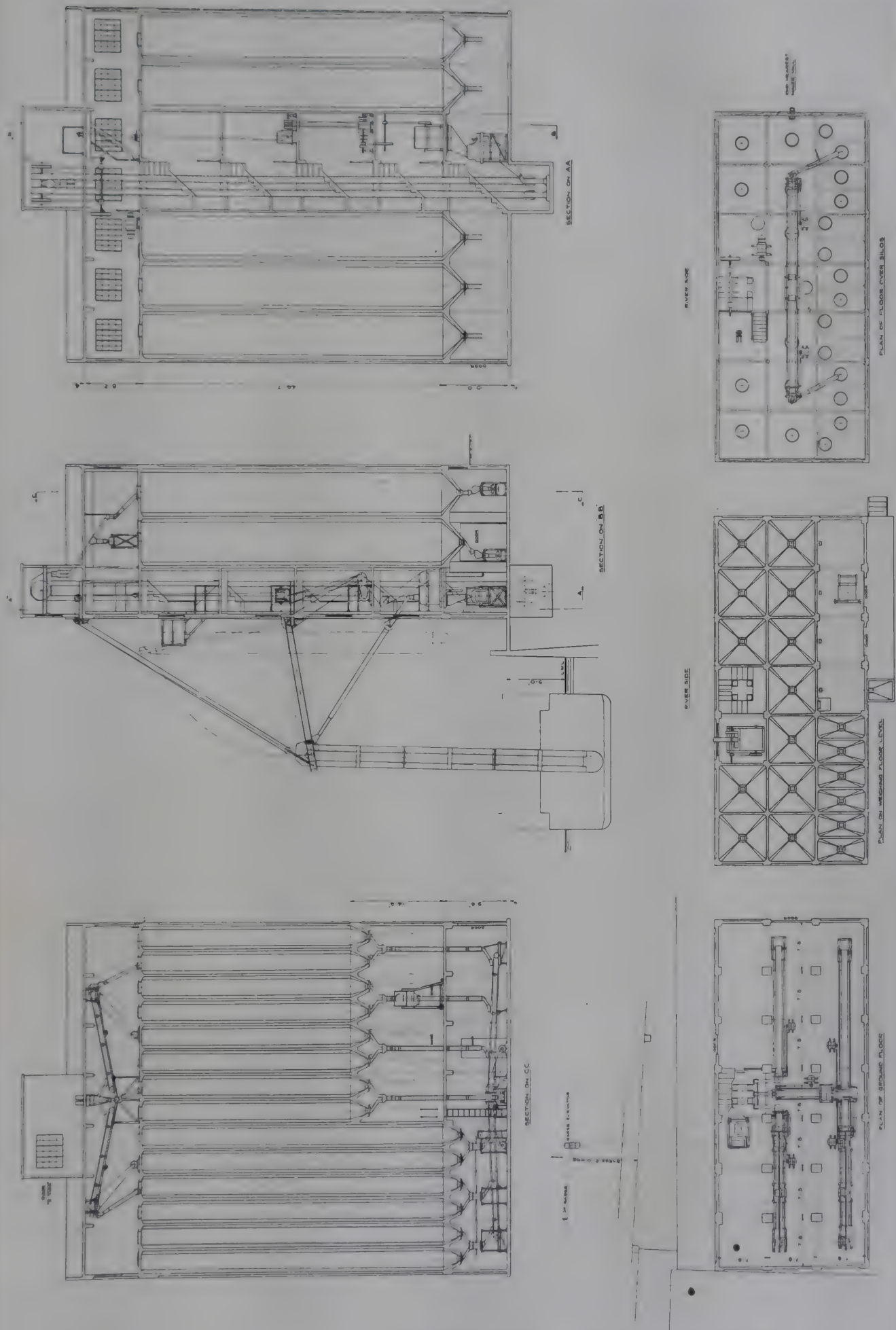


FIG. 1—A concrete silo, cross section and plan, capacity 1,000 tons.



FIG. 2 Another concrete silo, capacity 2,000 tons.

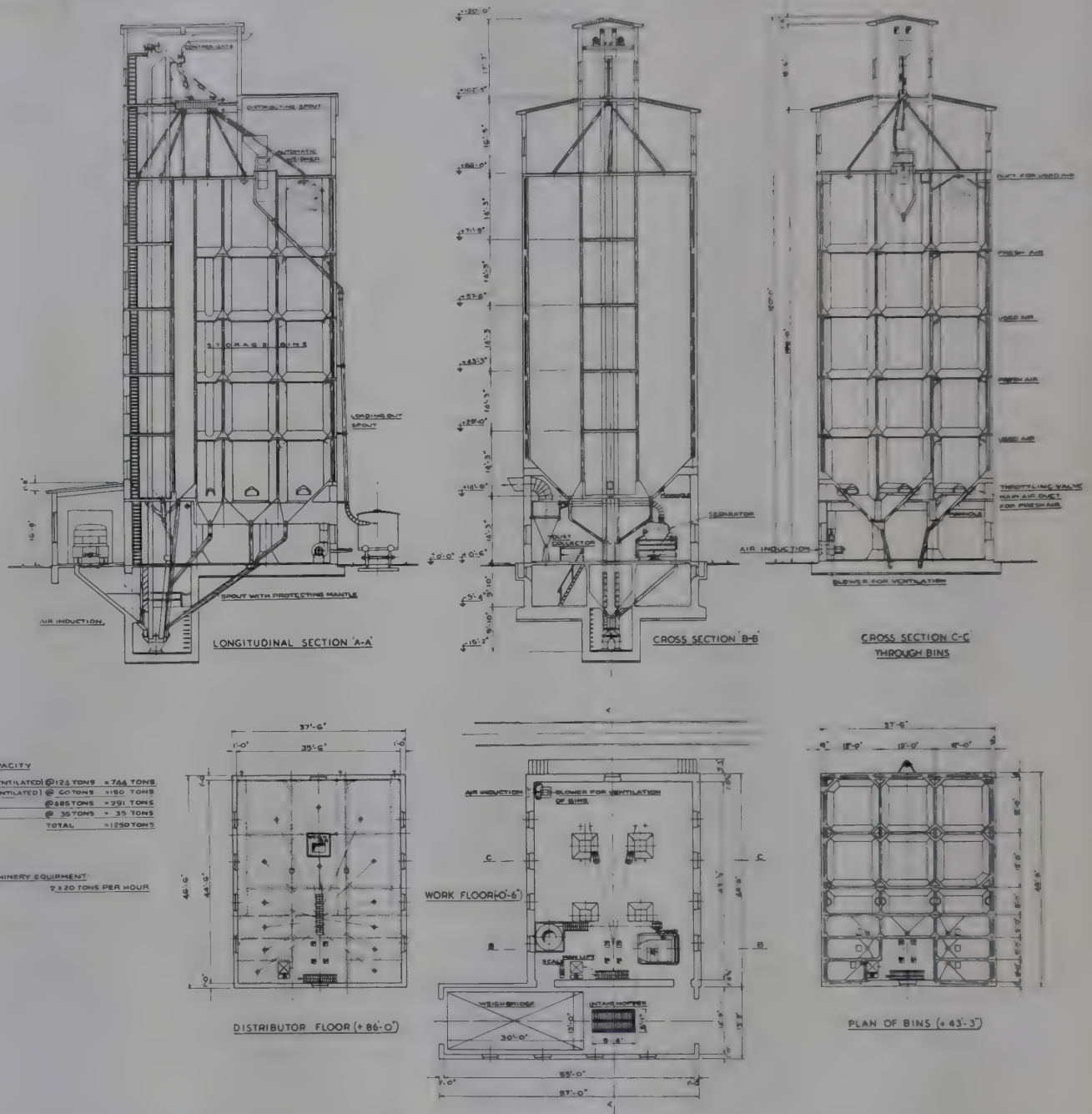


FIG. 4—A silo constructed on the improved ventilated bins system.

the cost of a silo on the total cubic contents of the buildings.

Existing Grain Stores.—It is generally accepted by most maltsters that properly harvested barley is all the better for remaining in stacks for two or three months, as was the old custom. If, however, it has been stacked too wet, the sooner it is broken down the better.

The usual practice has been to store barley on the upper floor of the maltings, sacks often being hoisted to the preliminary screens, dressing machines and barley drying kilns, with subsequent re-elevation and distribution for storage in comparatively small bin compartments in bulk or in heaps on the warehouse floors. The handling of barley stored in this way is a relatively costly operation, unless the bin compartments are self-emptying and mechanical or pneumatic handling equipment is provided for transporting the grain in a continuous flow.

Though the storage capacity in such cases is usually a matter of only a few weeks' supply, mechanization of an old malting is frequently difficult and sometimes impracticable.

There are, of course, numerous silos for storing barley in bulk, but the number of such stores is quite inadequate for receiving and storing the vast quantity now being produced in this country.

Design of Modern Silos.—It is almost universally accepted that the most economical method of storage of grain is in bulk, using bins of considerable depth.

The operations of handling and receiving in bulk can be performed with little or no labour, whereas handling in sack involves comparatively high labour charges, as there is no commercially successful method of handling and storing sacks of grain entirely by automatic means.

There are three orthodox types of construction in general use, each of which, however, is subject to variation with respect to combinations of materials used in construction. The three main types are:—

- (a) Reinforced concrete silos.
- (b) Timber silos.
- (c) Steel silos.

Each type possesses its own particular advantages.

(a) *Concrete* is adaptable to any size and shape of bin. It is extremely economical

with bins of large capacity, up to 30 ft. in diameter, such bin compartments holding as much as 1,200 tons. On the other hand, it may be adapted equally well to bins of alternative size and shape, such as square, hexagonal or octagonal. A silo of this type may be regarded as a permanent structure, requiring the minimum of maintenance.

With regard to capital cost, before 1939 a reinforced concrete silo would probably cost from 15 to 20 *per cent.* more than a similar silo in steel or timber. Under present circumstances, however, the cost of a reinforced concrete silo and a timber silo might be assumed to be much the same, whilst a silo of steel construction might be expected to show some slight economy in initial cost.

Before being filled, it is essential that the bins of a reinforced concrete silo should be thoroughly dried out, but the structure may be regarded as providing storage with perfect protection against weather and vermin, and is also regarded as being immune from fire risk.

Fig. 1 shows the interior of a silo of 1,000 tons capacity, suitable for storing native grain. This silo is of concrete construction, built on a piled foundation. The foundation loading for a grain silo, as previously mentioned, usually varies between $1\frac{3}{4}$ and $2\frac{1}{2}$ tons *per sq. ft.*, and unless the bearing capacity of the ground is suitable, a piled foundation is, of course, necessary. Fig. 2 illustrates the exterior of another concrete silo, also used for storage of native grain.

It will be seen from these figures that the building forms a permanent monolithic structure. The columns, walls and beams are reinforced with steel, and in order to ensure complete protection against the weather, the bin walls are usually not less than 5 in. thick. The lower portion of the bin hoppers is usually of steel, but apart from this, the whole structure is of reinforced concrete throughout, including walls and floors in the receiving house.

(b) *Timber* is the principal material used for construction of country silos in the North American Continent, both in the United States and Canada, largely because timber is the cheapest material available. It has been extensively used elsewhere, especially where suitable timber supplies are available.

In this country, silos having bins of laminated timber construction have been

favoured in the past by many brewers and millers, partly on account of their lower first cost, but mainly because it has been generally accepted in these industries that bin walls of timber are not subject to sweating, and are therefore eminently suited for the storage of their grain, particularly if home grown. The preference which has arisen in favour of timber, however, is more likely due to the fact that timber bins are far from airtight, and therefore very slight air circulation occurs in and around the bins, and even through the bin walls. Except under extremely humid atmospheric conditions, this will have a slightly beneficial effect in keeping the grain in wholesome condition. On the other hand, conditions of high air humidity are likely to have a harmful effect, which, by virtue of the type of construction, cannot be prevented.

With respect to maintenance, a timber silo requires appreciable up-keep, particularly the external sheeting which is liable to let in the weather if not periodically repaired or replaced. Consideration must also be given to the extra fire hazard of a timber silo, and risk of the timber harbouring or becoming attacked by insect pests.

In a typical silo of timber construction, the superstructure is built on a reinforced concrete piled foundation. The entablature of the silo, that is, the framework supporting the bins, is of structural steel, and the bin walls themselves are built in laminated timber on the steel entablature.

The timber work should be well seasoned white pine or timber of equal quality, planed on all sides, the walls being of varying thicknesses from about 4 in. at the bottom to 2½–3 in. at the top, depending on the dimensions of the bin compartments themselves.

Timber posts resting on the entablature are secured to the corner of the bin walls, and support the upper floor and roof. This construction is necessary, as the laminated timber forming the walls is subjected to a certain amount of compression and expansion as the bins are filled and emptied. It will be appreciated that the walls of the bins themselves are quite independent of the main framework of the structure itself.

Externally the bin timbers are protected from the weather by galvanized iron or asbestos sheeting or other suitable covering, and these sheets are secured to the timber

posts; that is, they are quite independent of the walling of the bins.

(c) *Steel plate* tanks form excellent containers for grain in countries where the atmosphere is dry; under such conditions they may be regarded as clean and durable, and under normal circumstances are cheaper in initial cost than reinforced concrete. Under present conditions, however, and with modern methods of concrete construction, the saving in initial cost is comparatively small.

The maintenance cost of a steel silo is considerable, as periodical painting of the whole structure is necessary, and a careful watch has to be kept on the bottom course of plating connected with the curb angles, where water is liable to lodge. It is generally considered that the life of a steel plate silo should be regarded as about 20 years. For the foregoing reasons silos of steel construction have not been generally adopted in this country.

A further disadvantage of steel construction is that, being a good conductor of heat, any change in atmospheric conditions is immediately transferred to the grain adjacent to the outside walls, a point which is particularly important if the grain has not been dried out to the desired moisture content. On the other hand, steel silos are fireproof and vermin proof and require no additional protective covering. They can also be constructed to suit all normal requirements with regard to size. From the point of view of erection, they have a distinct advantage compared with reinforced concrete or timber silos, as they can be constructed in a comparatively short time.

Steel tanks are usually supported on a steel framed structure or concrete entablature, with reinforced concrete foundations. Alternatively, the plates forming the cylindrical walls may be continued down to ground level, and the hopper suspended within at the desired height.

Storage bins of steel construction, as distinct from a complete steel tank silo, can be frequently used with advantage, and in preference to other types, for providing necessary reserve storage incidental to processing. The storage required in such cases is usually small, and is located within an existing building which provides necessary weather protection.

Having considered in general terms the merits and demerits of the main types of

TABLE II

COMPARATIVE QUANTITIES OF MATERIALS REQUIRED FOR A SILO OF 750 TONS CAPACITY CONSISTING OF 19 BINS IF CONSTRUCTED IN REINFORCED CONCRETE, TIMBER, OR STEEL

Type of Construction.	Materials.			
	Steel.		Timber.*	Corrugated sheeting.
Reinforced concrete	Superstructure	54 tons	20 standards each of 160 cu.ft.	15 squares each of 100 sq.ft.
	Foundation	18 tons		
	Total	72 tons		
Timber	Superstructure	66 tons	57 standards	160 squares
	Foundations	18 tons		
	Total	84 tons		
Steel	Superstructure	320 tons	4 standards	45 squares
	Foundations	18 tons		
	Total	338 tons		

* Calculation based on timber shuttering being used three times over.

construction, it is interesting, especially under present conditions of restricted supplies, to compare the quantities of materials used in the construction of these types (Table II). These quantities have been taken out and analysed in the case of a typical silo, reference Fig. 3, of 750 tons capacity, consisting of 19 bins.

Machinery Equipment.—The subject of machinery equipment is a comprehensive one in itself. We are here concerned chiefly with the machinery required for an inland silo used primarily for handling native grain, as distinct from that used in a large port silo handling mainly imported grain.

Since the grain is stored in bulk, it may be handled either mechanically or pneumatically, with complete elimination of manual transport. The tendency in modern design is towards automatic control, and a completely automatic silo is already an achievement of the past. The problem of mechanization in the maltings has been the subject of a paper by H. A. D. Cherry-Downes (this *Journ.*, 1948, 208). The handling operations in the silo should be considered in relation to those in the maltings as a whole.

As far as the silo is concerned, these operations include the receiving plant, which may be required to deal with grain arriving in sack or bulk by road, rail or inland waterway; the capacity is usually between 15 and 50 tons *per* hour, and the plant comprises the

intake elevator, weigher and separator for rough cleaning. At present, native grain is transported mainly in sack, but future development in methods of transport will inevitably result in more extensive use of bulk transport both by road and rail on the journey from the farm to the malting or mill, and this factor should not be overlooked in planning new storage accommodation.

In order to ensure a rapid turn-round of large bulk lorries of 12 to 15 tons capacity, without corresponding increase in capacity of the handling plant, it is necessary to provide a dumping hopper approximately equal in capacity to the largest vehicle, so that the grain may be unloaded rapidly by opening the discharge gates, and the vehicle released in a matter of a few minutes.

In addition to intake, each silo should be equipped with means of turning-over the grain from bin to bin.

The handling to and from the barley kilns or drying plant, and of the dried barley to the steepers, are operations to be considered in connection with the silo design.

In view of the malting process being essentially a batch as distinct from a continuous process, and the rather extensive area frequently occupied by the premises, pneumatic handling has a special appeal, as a single pneumatic pump unit may be installed to perform a multiplicity of operations with corresponding economy in initial cost of plant.

Recent Developments in Silo Construction.—Reference has been made to the nature and magnitude of the problem of providing additional storage in this country, particularly for native grain.

To the brewing, milling and other industries concerned with such grain as a basic raw material, the essential conditions to be complied with in providing additional storage are, first, preservation of quality, *i.e.*, safe storage, and second, the adoption of constructional methods suited to our national economy.

British Intelligence Objectives Sub-Committee's *Reports*, Nos. 439 and 733, refer to the subject of grain storage in Germany, where over 90 *per cent.* is home produced.

The problem of grain storage in the United Kingdom has much in common with conditions which have prevailed in Germany for many years, and it is interesting to note that these *Reports* refer to the wide use of forced ventilation as fairly common practice in central Europe.

Many of the technical aspects of forced ventilation in grain silos are discussed by T. A. Oxley in *The Scientific Principles of Grain Storage* (Liverpool: Northern Publishing Co., Ltd., 1948).

There is briefly described below one possible solution to meet the present problem of grain storage, which complies with the essential conditions mentioned above, namely, safe storage and a method of construction suited to the national economy. This solution has the special virtue, where the malting industry is concerned, that it involves a minimum addition to existing plant for sweating barley.

This is an improved system of bulk storage in deep bins provided with means of uniform and controlled ventilation, either with fresh or conditioned air. As previously mentioned, this principle of forced ventilation has been employed in central Europe just prior to and during the second world war, and opportunity has therefore been provided for testing its effectiveness over a long period and on a practical commercial scale.

The system provides by simple means uniform and intimate contact of every grain stored in a deep bin with a controlled current of fresh or conditioned air.

The forced air circulation is introduced into main air ducts which form an integral part of the bin construction, the fresh air being

introduced into individual bins by means of throttling valves; from the main inlet ducts, the air passes upward through vertical shafts, one to each bin compartment of the silo, and then to a series of horizontal ribs arranged one above the other at intervals and forming a complete ring round the bin wall. From all these horizontal ribs surrounding the wall, fresh air enters and passes through a predetermined depth of grain, cooling the grain in so doing, and escaping through similar horizontal ribs also completely surrounding the bin walls and arranged alternately with the air inlet rings. The horizontal exhaust ribs are also connected with independent vertical air shafts, one to each bin compartment, which ultimately connect with the main exhaust duct.

The resistance to air flow through the grain at the bottom of the bin is greater than that through a corresponding depth near the surface, and to ensure uniform treatment of the grain throughout the depth of the bin, the spacing of the horizontal ribs between which the air passes is gradually decreased with the depth in the bin.

Normal ventilation as described would involve a forced ventilation at the rate of approximately 40 cu.yd. of fresh air *per* hour *per* ton of grain in the bin; the static resistance of the system is very low (in the neighbourhood of 4 in. water), and the power required by the fan to ventilate simultaneously four bins each of 50 tons capacity would not exceed about 5 h.p.

For dealing with grain in poor condition, provision may be made in a group of smaller bins for a more intensive ventilation, namely 80 cu.yd. of air *per* hour *per* ton. In such a case the ventilation would be by conditioned air, the air passing horizontally through the grain for a shorter distance. Under these conditions, a fan of the size used for normal ventilation would successfully treat four bins, each of 25 tons capacity, simultaneously. The method of application of this system of ventilation is illustrated in Fig. 4.

The fan or blower is usually located on the working floor under the bins of the silo, and can be combined with an air purifier.

Additional flexibility is provided by introducing two blowing units, one of which can circulate conditioned air through the grain in case of necessity.

It is recognized that the average moisture-

content of the atmosphere in this country for most of the year exceeds the average moisture content in many areas of the Continent of Europe, where similar storage systems have been in use for a number of years. The conditions for safe aeration of grain, however, have been established on a scientific basis, and fresh air under any atmospheric conditions may be passed through the grain, providing the temperature of the fresh air is 7°-9° F. lower than the temperature of the grain in the bins. The principal object of the aeration of grains is the avoidance of self-heating, which may arise either from respiration of the grain itself or from the activity of moulds. It has been established that mould growth develops in the presence of air with 80 *per cent.* relative humidity, and therefore, one of the conditions of ventilation on this system is to avoid the use of air which after ventilation has a relative humidity exceeding 75 *per cent.* Simultaneously, moisture may be removed from the grain by ventilation, thereby combining the provision of storage with a limited drying action. The relative humidity of the fresh air as it passes through a mass of warm grain is lowered, and therefore its capacity for removing moisture from the grain is increased; in other words, grain can be ventilated in safety, providing the waste air as it leaves the exhaust ducts from the bins does not show more than 75 *per cent.* relative humidity.

In the first place, the temperature of the grain has to be measured, and this can be done with great simplicity by electric resistance thermometers. All up-to-date silos, particularly those required for storing barley for malting, should be so equipped.

The temperature of the grain within the bin is measured by suspending electric resistance thermometers at intervals of say 10 ft. down the centre of each bin, the thermometers being suspended within a tube or by wire secured from the bin top. A small electric current is passed through each thermometer, and as the electric resistance increases with the temperature, a galvanometer to measure this resistance can be so calibrated as to indicate the actual temperature of the grain in degrees Fahrenheit.

All the thermometers are wired to a central indicating board, over which the temperature dial is fixed, so that in a silo of 20 bins, each having three thermometers, a panel with 60 press buttons is provided, and the tem-

perature of the grain at any position of any bin can be determined immediately. In this way, heating of the grain can be detected by taking periodical readings. If it is necessary to ventilate, the temperature and humidity of the fresh air should be measured, and the temperature and moisture content of the exhaust air should also be taken during ventilation.

If grain with a temperature of 60° F. is ventilated with fresh air at 50° F., no harm can result. Even if the fresh air entering is 100 *per cent.* saturated, the induced air is warmed within the grain to such an extent that the exhaust air reaches at the most 75 *per cent.* saturation on leaving the bin. The drying effect naturally increases as the relative humidity of the air decreases. Should the temperature of the fresh air be, say, 54° F., and the grain temperature 60° F., that is, a difference of 6°, then the relative humidity of the fresh air used for ventilation should not exceed 80 *per cent.* in order to ensure that the leaving air does not exceed 75 *per cent.* saturation.

It may be necessary in the warmer months to confine ventilation by fresh air to the early morning and evening in order to comply with these conditions of ventilation, namely, that the temperature differences between grain and air should be 7°-9° F. under conditions of high humidity.

To meet the abnormal humid conditions of this country, and the high moisture content of grain, the system provides for the use of conditioned air when necessary. The simplest method of achieving the desired results is to pass the air through a heating element immediately before it enters the main air inlet ducts. The heating elements may be electric, gas or exhaust from diesel engines, or whatever waste heat is available. The air is heated to 80°-90° F., and the ventilation continued with air at this temperature for a period of about two hours. During this period the grain will ultimately acquire a uniform high temperature, and moisture will be removed. Having established a substantial temperature difference between the grain in the bin and the outside atmosphere, this pre-treatment by conditioned air is then followed by passing fresh air through the grain. This may now be done in safety; a certain amount of moisture will continue to be removed, and finally the grain will be cooled down within a few degrees of the outside air temperature.

Grain which has been pre-warmed in this way acts as a medium for lowering the relative humidity of air in its passage through the grain mass, thus enabling the operations of drying and ventilation to be performed in safety. The problem of high atmospheric humidity so prevalent in this country is thus overcome, and as a result of this treatment the grain is kept in sweet condition free from heat.

The principle of the design is the construction of the bin walls using reinforced concrete masonry without timber forms. For this purpose special concrete blocks are made in standard sizes, which can be fabricated on the site by specially designed block-making machines.

The blocks are constructed with semi-circular furrows on the upper and lower faces, and within the circular spaces formed in the wall steel rods of $\frac{3}{16}$ or $\frac{1}{4}$ in. diameter are employed for reinforcing purposes.

The blocks are hollow and have smooth sides, so that no internal treatment to the bin face is necessary after the walls are constructed, and there is no risk of lodgement of grain.

At the bin corners special blocks are made to permit the interlacing of the horizontal reinforcing rods.

Special precast blocks are also made for the saddles which form the air ducts in the bin hoppers, also the ribs forming the horizontal air ducts around the bin walls, and the fresh and exhaust air vertical shafts within the bins.

All these precast blocks are made in steel moulds, and are therefore perfectly uniform in size.

The effect of the projecting saddles forming air ducts within the bin is to increase the vertical loads on the walls and to reduce the lateral pressure within the bin. This reduction, in a deep bin, amounts to approximately 35 *per cent.* compared with the bin wall of normal construction.

The use of vertical steel reinforcement in the bin walls is entirely eliminated, thus saving some 30 *per cent.* of the total steel reinforcement required. The effect of the vertical ventilation shafts is to act as a stiffening to the walls.

The exterior walls of the silo will be of precast blocks constituting the bin walls,

TABLE III

COMPARATIVE QUANTITIES OF MATERIALS REQUIRED FOR A SILO OF 750 TONS CAPACITY
CONSISTING OF 19 BINS IF CONSTRUCTED IN REINFORCED CONCRETE, TIMBER, STEEL
OR ON THE VENTILATED SYSTEM DESCRIBED

Type of Construction.	Materials.			
	Steel.		Timber.*	Corrugated sheeting.
Reinforced concrete	Superstructure ..	54 tons	20 standards each of 160 cu.ft.	15 squares each of 100 sq.ft.
	Foundations	18 tons		
	Total	72 tons		
Timber	Superstructure ..	66 tons	57 standards	160 squares
	Foundations	18 tons		
	Total	84 tons		
Steel	Superstructure ..	320 tons	4 standards	45 squares
	Foundations	18 tons		
	Total	338 tons		
Ventilated system described	Superstructure ..	40 tons	10 standards	15 squares
	Foundations	18 tons		
	Total	58 tons		

* Calculation based on timber shuttering being used three times over.

with an exterior wall of brick masonry built so as to produce a cavity wall. The outer brick wall can be of facing bricks, or alternatively common brick with an external rendering. The air cavity between the bin wall and the outer brick wall provides a very desirable insulation and a completely effective shield against all weathers.

The main columns supporting the bins, hopper bottoms, floor slabs and roof construction, are designed generally in accordance with usual practice for reinforced concrete silos, though a roof slab of reinforced hollow tiles can be used as it provides a better insulation.

This system of construction, besides providing a very important saving in the use of steel, also provides an equally important saving in the use of timber forms. There is a further saving in time required for erection, as the period normally required for installation of the sliding mechanism and working

platforms for the normal type of construction is eliminated. Instead, a moving scaffold is provided within the bin, which has been specially devised to suit this type of construction.

The amount of steel and timber required for a typical silo as previously illustrated in Fig. 3, and constructed in accordance with this system, can be compared with that used in silos of reinforced concrete, timber and steel, constructed in accordance with ordinary methods (Table III).

It will be seen from these figures that the system described gives a saving of about 30 *per cent.* of steel and over 50 *per cent.* of timber compared with a concrete silo of orthodox type. It can be appreciated, therefore, that a ventilated concrete silo of the type described combines all of the advantages of the other types and is eminently suited to conditions prevailing to-day.

MEETING OF THE MIDLAND COUNTIES SECTION HELD AT THE
WHITE HORSE HOTEL, CONGREVE STREET, BIRMINGHAM, ON
THURSDAY, 22nd SEPTEMBER, 1949

MR. B. WOOD in the Chair.

The following paper was read and discussed:—

THE 1949 BARLEY HARVEST

By E. P. WRIGHT

The barley harvest has been extremely variable in quality and yield. Both autumn and spring seed beds were ideal, and in spite of the very dry summer, the yield over the whole country has been above normal. The harvest weather was ideal, and the increase in the number of combine harvesters enabled the crop to be gathered in record time. There should be sufficient barley of reasonable quality for the brewing trade.

VARIATION in the quality of barley this year appears to be greater than for several years, and climatic conditions, time of sowing and manurial treatment are the chief factors responsible for this variation. The dry autumn and winter of last year enabled farmers to drill a considerable acreage of barley before Christmas, and owing to the dry winter, land was also in excellent condition for early spring drilling, and drilling was completed much earlier than usual. The autumn-sown and early spring-sown barleys grew satisfactorily and the strong growth and

vigorous root development enabled them to withstand the later very dry weather, with growth helping to prevent excessive moisture evaporation by covering the land well, and the good root development providing the necessary moisture to keep the corn healthy and ultimately to have a good yield. The later-sown barleys, however, did not produce the same root development and the corn was not sufficiently developed to prevent excessive moisture evaporation in the later dry weather, and consequently died before reaching maturity, the corn being thin and

steely and highly nitrogenous and generally unfit for malting for brewing purposes. Yields have been most variable; they have been very good to very poor, but probably a little higher than normal over the whole country.

The foregoing applies generally to all districts, and in all districts there is the same great variation in quality and yield. Most of the corn is white or creamy in colour, but there are some barleys quite yellow, and which cut quite mellow, due no doubt to having rain which other districts did not get. During the 12 months ending August, there were, speaking generally, 6 in. less rain than during the previous 12 months, and the whole of this deficiency occurred in the critical months of April to August, which period was exceptionally dry.

The weather at harvest was very fine and the corn was harvested in record time with a very low moisture content of 12 *per cent.*, and there has been no trouble with combined barleys deteriorating in transit due to high moisture. Also the deterioration of barley in farmer's barns which often occurs with high moisture content combined barleys has not been experienced this year, as the moisture of the corn is nearly as low as the recognized safety maximum for storage.

Nevertheless, it is necessary to dry, or, to use an old expression, "sweat" the barley on arrival, and rest it thereafter to overcome dormancy, as freshly harvested barley will not germinate to its fullest capacity until it has had either a natural sweat in the stack or an artificial sweat by drying.

The nitrogen content of the season's barley is also very variable, but the variation is not so great in the qualities which concern brewers. The few mellow barleys, mostly from Norfolk, have a nitrogen content of 1.1-1.2 *per cent.*, and the thin immature barleys which died prematurely have as much as 1.8 *per cent.* of nitrogen. However, the qualities which have generally been purchased for brewing purposes from the southern half of the country have a nitrogen content of 1.3-1.5 *per cent.*, and those from the northern half 1.4-1.6 *per cent.*

It is known that in recent years there have been various types of barley introduced into this country by importation or hybridization. The varieties Abed Kenia, Abed Maja and Freja are Danish barleys grown for their higher yields, and early maturing, and

Pioneer and Earl are hybrids grown for similar reasons. The high price of millable barley fixed by the Government has caused these barleys to find favour with farmers who have no need to think about quality; for with the mechanization of farming, they are enabled to complete their harvest quickly, and the high yield and generally higher moisture of the combined grain, with no expense of stooking and stacking, and no loss by vermin, probably gives them as much profit as if they had produced good malting barley at a higher price, and without so much trouble or risk. In this connection it is interesting to note that for the 1950 crop the price of milling barley has been reduced by 7s. *per* qtr. (448 lb.) and the price of wheat increased by about £1 *per* qtr. This will undoubtedly mean a lower acreage of barley next year.

The increase in the number of combine harvesters caused a glut of barley on the market, far more than the trade could absorb, with the consequence that the Ministry of Food was an extremely heavy buyer and possibly took delivery of some barley the trade might be glad to have later on, for it is problematical what quality of barley has gone to stack, and the quantity stacked is much less than usual. Since the advent of the combine, it is a fact that the average quality of barley stacked is not so good as what the trade purchases at harvest time when there is a great selection, and it is certain that if the average quality in the stack is only as good as the average quality already dealt with by the trade and the Ministry, then that average cannot be good. Most consumers, however, have now increased their intake capacity of barley, and it may be that there will be sufficient useful barley in stack to fill the balance of the brewing and malting requirements, leaving the rest to be taken by the Ministry.

There has also been a tendency for farmers to instal their own silos with or without aeration and drying plant. This, in some respects, is a good thing, but the correct storing of barley for malting purposes is of prime importance, and it is essential, particularly in seasons not so dry as this, that barley purchased a few weeks after harvest should be accepted subject to satisfactory germination; even a germination test is no criterion of the quality of the corn, for it is possible, and probable, that barley can suffer

deterioration in vitality with incorrect storage, even if a germination test may show the embryos not to be dead. If this paper receives any publicity in agricultural journals it is desired to emphasize the point that where farmers store their own barley, there should be constant circulation or turning of the corn to aerate it, to keep the temperature down, and to get rid of the deleterious carbon dioxide gas which corn produces in bulk. In cases where farmers dry their barley in the hope of selling it for malting purposes, they should drop their drying temperatures in accordance with the moisture content of the grain; even at 110° F. with high moistures, damage can be done to embryos for, although this temperature will not kill them, it can impair their vitality if the moisture content is high, and a weakly growth is nearly as bad for a maltster as no growth at all.

Before the advent of the combine harvester the small quantity of corn threshed out of the field was dealt with quickly, and the majority of the crop was threshed gradually from stack and damage by heat was easily discernible to the expert, but it is not always easy to discern damage to barley which has been in a farmer's barn for a few weeks, and it is dangerous to buy barley except immediately at harvest, or guaranteed fresh threshed from stack. It is, perhaps, too much to expect farmers to look after threshed barley on their farms as well as a maltster or brewer-maltster would, but the danger is much less in a dry season such as 1949, when most barleys have a very low moisture content; actually, however, there are not many harvests as dry as this year's. The rapid tetrazolium test enables a determination of the live corns to be quickly made, and this is often of great help, although it is not possible to assess evenness of growth of the embryos; also the method is somewhat

laborious, involving the cutting through of the embryos of 300 corns for each sample tested. Troublesome though this method of testing is, it is worth while to avoid having a deteriorated consignment of barley put in a large bulk.

This season there is a gentlemen's agreement between the interested parties that any barley bought for beer brewing purposes, should not be less than 103s. *per* qtr. to the farmer, an increase of 10s. *per* qtr. over the price for millable barley. This was in effect designed as a brewers' subsidy to farmers, but in many cases it has acted awkwardly for, if there had been a free market, it would have been possible at harvest time to purchase quite useful barley on its merits below 103s., and very many consignments have been offered at less than 103s. which could have been bought for mild ale malts had it not been for the agreement. Such barley has been sold to the Ministry at millable price, except for the quantity which has been bought for malting for other purposes than beer brewing, and consequently the farmers concerned have taken millable price for such corn, whereas they would, in a free market, have made between 93s. and 103s. according to quality.

No doubt the question of increased barley storage is of interest to many consumers, and it may be of interest to mention two large grain silos which are being erected in what is thought to be a cheap way. The Stent type of storage bin is being used. This is a circular bin of pre-cast concrete blocks, tongued and grooved, and is much cheaper than steel or reinforced concrete, the cost being about £6 *per* ton storage space against two or three times as much for the other types. These figures do not include the cost of the plant for filling, emptying and for re-circulation.

COMMUNICATIONS

MOISTURE DETERMINATIONS IN BARLEY AND MALT

By B. TROLLE, D.Sc.

(Research Laboratory, Carlsberg Breweries, Copenhagen)

Moisture is defined as the loss of weight which a substance undergoes by evaporation, during a reversible process of drying to practically constant weight, under such conditions that it does not suffer detectable alterations of any significance other than reversible loss of moisture. A reference method, conforming as closely as possible to the principle of this definition as present-day biochemical knowledge will allow, has been worked out; it involves drying the finely-ground substance to practically constant weight *in vacuo* (1-10 mm. Hg) over phosphorus pentoxide at approximately 40° C. It is assumed that this reference method can be carried out in any laboratory. Any routine method for moisture determination may be adjusted to it, and there is thus a wider choice of routine methods, even though some of these cannot be themselves standardized owing to their more or less empirical nature.

INTRODUCTION

Determination of the moisture content in barley and malt as well as in cereals generally is a very important and a very old problem. In spite of being copiously treated in the literature over a long period the problem is still not resolved. On the contrary, there is a great diversity of methods applied—both between the various branches of cereal chemistry and even within each individual branch. This diversity is unsatisfactory in daily work, and the perpetual discussions on methods are time-wasting. A series of dry-matter balances connected with the work at Carlsberg Pilot Brewery has led further into the problem of moisture determination over a period of more than three years. Thereby an extensive knowledge of the earlier literature has been gained, in which are to be found several eminent experimental works. It has been found that, in spite of all, the opinions are not so different as the varying methods would suggest, and there should thus be an excellent foundation for an agreement about common lines. This opinion has led to the present renewed discussion of the problem.

From a theoretical point of view the development of biochemistry has not as yet made it possible to define what is to be understood by moisture in organic material. Whether the moisture under consideration should be divided into moisture of constitution and moisture of wetness; whether it should be more specifically named as moisture

of constitution, water of crystallization, water of swelling, capillary water and moisture of wetness; or whether it should be designated as physically bound water as opposed to chemically bound water [water of crystallization, hydration-water (water bound through hydrogen bonds), *etc.*], present knowledge of the structure and constitution of organic materials does not make it possible to account for which moisture is leaving during the process of drying, and it will be impossible to define which moisture should be comprehended by a moisture determination.

It is therefore necessary to give up the ideal of making the moisture determination theoretically secured, and instead endeavours should be made to approach the theoretical ideal from the practical side, *i.e.*, based on practical considerations. Through suitable development of these, there is a possibility of approximating, step by step, the theoretical aim by a different way. So, though less direct, the method is absolutely practicable.

From a practical point of view the first principle for moisture determination, or the definition of moisture, must have the following wording:—

By moisture is to be understood the loss of weight which the substance undergoes by evaporation during a reversible process of drying to constant weight, under such conditions that the substance does not undergo other alteration than a reversible loss of moisture.

Even this definition is too theoretical, and must be altered in the following way in order to form the foundation for the practical work:—

By moisture is to be understood the loss of weight which the substance undergoes by evaporation during a reversible process of drying to practically constant weight, under such conditions that the substance does not undergo *detectable* alteration of any significance other than a reversible loss of moisture.

By the expression "detectable alteration" the definition is made to comprise the recognition of the inadequacy of present-day chemical methods; at the same time the definition in future can be brought up-to-date corresponding with development, "detectable alterations" implying the practical indications on which to rely at any time, in order to secure in the best possible way (and step by step aiming at a perfectly valid guarantee), that the substance does not undergo other alterations than the reversible loss of moisture. A detectable alteration not associated with any alteration of moisture-character, nor—as a matter of course—with any loss of weight, need not necessarily limit the requirements for the free operation of the reversible drying; therefore the clause "of any significance" has been introduced, this to be interpreted with the necessary circumspection and in deference to the biochemical knowledge at a particular time.

The above definition contains only a principle for the moisture determination, and thus, supported by convenient lines of working, is able to function as a standard for moisture determinations. The principle will not become out-dated; it can still be kept in conformity with the development of chemical methods, and it will render future fundamental discussions unnecessary. The way used hitherto of standardizing a special method and a special apparatus does not seem correct, as far as conventional methods are considered. It may easily cause a check on development.

The standardizing of a principle gives the individual chemist, or the individual laboratory, a freer hand, but also a greater responsibility, than the standardization in details of a special method. It is possible to use the most correct method of reference for moisture determination available at a

given time; this, however, may probably be too protracted for utilizing in daily work, but fit for adaptation for any optional method proved to be the best in the daily work in the individual laboratory. The demands on a moisture determination must vary according to the tasks of the individual laboratory and the individual industry. In places it will be necessary to use speedy methods (1 hr., 2 hr., 3 hr., *etc.*); elsewhere this is not needed. In some places less accuracy is required, while a higher degree of exactness is demanded somewhere else. Roughly speaking, the daily method in an individual laboratory will be a private matter for that laboratory, chosen on its own responsibility, and irrespective of any demands made in other laboratories. The main point must be that all moisture determinations are adjusted to one and the same reference-method, practicable everywhere, the principle of which is not debatable, but which still allows the execution to be kept in conformity with development.

Also, the above-mentioned line of action for standardizing will facilitate international co-operation, special apparatus of fixed manufacture being unnecessary. Experience shows that ovens differ rather widely in the various countries, the differences, however, being of no fundamental importance. It is thus unreasonable, as well as hampering for the extension of a proposal, to claim particular types, applied as prescribed in certain standard proposals now in force.

Literature.—The following discussion of the literature is given only to show that for many years there was a conception for moisture determinations so evident, that it is easily classed under the principle for moisture determinations given here.

Riiber^{8,9} gave in 1891 and in 1897¹⁰ the fundamental principles for moisture determinations by drying. These principles were based on extraordinarily exact and careful experiments, and are perfectly valid even to-day. Briefly, Riiber's principles and results can be summed up as follows:—

"I call attention to the fact that for determining the point of time for the drying, at which this may be regarded as carried to an end, we have been working arbitrarily. For we estimate the drying procedure from two weighings of the drying substance, and when the last weighing which is perhaps performed two hours after the preceding one gives the same result, we have 'constant weight,' and the drying is complete.

"We have, however, not taken into consideration that it cannot be unimportant, if the substance has been drying only two hours or, for instance, 24 hours beforehand.

"The time of drying before the first weighing must be in a certain relationship to the continued drying until the second weighing, and therefore it is fixed as a condition for the complete drying that the second drying must last as long as the first one in order to be able to conclude from the two weighings that the drying is complete."

As regards the most favourable conditions for wort-extract, beer-extract and maltose, Riiber states:—

"... a temperature of 80° C. under vacuum (about 20 mm. Hg) for two days appears to be sufficient both for the drying of wort and of beer-extract, so that subsequently I have always used this temperature for scientific work; furthermore, the values found for beer-extract are quite well in accord with values found after one week at 55° C."

Ford and Guthrie,⁵ in 1905, discussed moisture determinations in malt. Their work was not nearly so comprehensive as that of Riiber, and so has not the same wide bearing, but their conclusion is of interest. They write:—

"The use of vacuum drying appliances at, say, 105–110° C. would undoubtedly yield results approximate to the truth, but these appliances are not in general use, and it is doubtful if the needs of the case justify the adoption of such expensive and troublesome apparatus."

Berglund,¹ mentioning some of the various methods used for moisture determinations, points out that they are unfitted to be standard methods owing to the fact that the drying temperatures used are generally at about 100° C. A decomposition of the most important constituent, the starch, takes place at this temperature. That starch is very difficult to dry appears from one of Berglund's literature references to Malfitano and Moschkoff,⁶ who have proved that starch becomes 28 *per cent.* soluble after five weeks' drying *in vacuo*. In his own experiments, Berglund defines moisture thus:

"... the evaporation loss to constant weight, when the substance is placed over P_2O_5 *in vacuo* (10 mm. Hg) at room temperature."

To accord with this definition, he has used a tight-fitting vacuum desiccator with relay-controlled heating unit. As regards barley, it was found after 60 days' drying in conformity with the definition that the weight from the thirty-fourth day was practically constant.

The outstanding work of Sair and Fetzer¹¹ was available to the present writer only after the war, at a time when his own experiments were already far advanced. Their determinations included the use of vacuum, and as the experiments described below are based on vacuum determinations it was a great pleasure and satisfaction to find the striking similarity of the vacuum methods employed and the results achieved. At the same time, Sair and Fetzer's clear results have enabled the present work to be brought to a provisional conclusion earlier than would otherwise have been possible.

Sair and Fetzer used finely-ground maize (40-mesh). The methods employed supplemented each other in a most outstanding manner, whereby the whole investigation is given an exceptionally convincing value. The following methods were employed:—

1. *Distillation* (direct water) with toluene, benzene and xylene (duplicates agree within 0.10 *per cent.*). The procedure followed was essentially that outlined by Bidwell and Sterling,² with the exception of specially designed apparatus (*cf.* the method given by A.O.A.C.).

2. *Oven drying* (indirect water) under vacuum and atmospheric pressure (duplicates agree within 0.03 *per cent.*). The standard A.A.C.C. moisture dish was used (55 × 15 mm.) with a sample of 4.5–5.5 gm.

3. *Drying in vacuo according to De Bruyn* (indirect water). This method resembles that of Riiber and dates from 1894. Later, the method was greatly improved by Cleland and Fetzer.⁴ In principle, the method allows prolonged drying *in vacuo* at low temperatures in presence of P_2O_5 . The improved apparatus consists of two pear-shaped 250-ml. glass flasks with standard taper joints, connected by a glass tube of approximately 3 cm. diameter and 30 cm. in length, with a glass cock for vacuum. One of the glass flasks is filled with the material to be dried, and the other with P_2O_5 . The glass cock closed, the apparatus is able to keep vacuum at 0.1 mm. for long periods. 10–15 gm. of substance is weighed into a tared De Bruyn flask, and the drying takes place at 38° C. and 0.1 mm. Hg. Duplicates agree to within 0.03 *per cent.*

4. *Extraction*.—The principle of this method is resolution of the sample into stable and unstable fractions, with special

treatments suitable for each fraction. Duplicates agree to within 0.06 *per cent.*

5. *Studies of Reversibility.*—These involve the assumption that water-holding capacity is a physical constant, if decomposition or

considered to be unchanged by the drying process.

A survey of Sair and Fetzer's results for the various methods is given in Table I. They conclude:

TABLE I
SUMMARY OF MOISTURE DATA FOR 40-MESH MAIZE

Moisture method.	Conditions.	Length of drying, showing period of essential constancy (hr.).	Final apparent moisture value (per cent.).
Distillation	Benzene—b.p. 80° C.	68–92	11.51
	Toluene—b.p. 110° C.	20–45	11.51
	Xylene-toluene—b.p. 120° C.	16–45*	11.70
	Xylene—b.p. 140° C.	16–35	11.74
Oven drying (Weber oven)	Vacuum—70° C.	46–110*	11.55
	(0.2–2.5 mm. Hg) 80° C.	65–110*	11.77
	100° C.	48–72*	12.06
	110° C.	16–32*	12.05
	Air—100° C.	45–130	10.84
	Air—P ₂ O ₅ —100° C.	20–130*	11.77
De Bruyn	Corn alone—38° C.	650–850	11.48
	Corn alone—50° C.	additional 100	11.47
	Corn with alcohol—38° C.	650–850	11.46
	Corn with alcohol—50° C.	additional 100	11.46
Extraction method ..	(1) Ether extract—75° C.—N ₂	1–3	11.60
	Ether-insoluble—100° C.— <i>in vacuo</i>	18–36	—
	(2) Ether extract—75° C.—N ₂	2–6	—
	Ether-insoluble—100° C.— <i>in vacuo</i>	24–48	11.56
Reversibility	(1) Corn pre-dried—100° C.— <i>in vacuo</i>	—	11.48
	(2) Corn pre-dried—110° C.— <i>in vacuo</i>	—	11.47

* Complete constancy not obtained.

chemical alteration has not occurred. Urquhart and Eckersall¹² and Pidgeon and Maass⁷ have shown that cellulose containing 1 *per cent.* of moisture may lose it reversibly. For their studies of reversibility in cereals, Sair and Fetzer employed the technique prescribed for cellulose. A control sample of the cereal or feed was dried under conditions which eliminated any possibility of decomposition and which permitted the material to retain about 1 *per cent.* of moisture (vacuum oven, 40° C., 340 hr.). Other samples were dried for various periods under drying conditions sufficient to remove the total moisture. These samples were then placed in proximity to the controls, under which conditions the more completely desiccated samples reabsorbed moisture. Samples which absorb sufficient moisture to yield a final apparent moisture content equal to the control were

Distillation of maize with benzene or toluene, yielded similar results (11.51 *per cent.*), while the use of solvents of higher boiling point resulted in higher moisture values. This increase was accompanied by an intense red colour formation in the corn.

The oven methods yielded widely varying results, which ranged from the low value of 10.84 *per cent.* for the air oven at 100° C. (130 hr.) to the high value of 12.05 *per cent.* for the vacuum oven at 100° C. (72 hr.). It was demonstrated that the maize dried in the air oven still contained moisture, as shown by the fact that when the material was placed in the air oven at 100° C. over P₂O₅, a moisture value of 11.77 *per cent.* was obtained.

Maize can be dried to constancy at low temperatures (38°–50° C.) *in vacuo* over P₂O₅.

(De Bruyn) to yield a moisture value essentially equal to that obtained by the benzene or toluene distillation method (within 0.05 per cent.).

By first extracting maize with diethyl ether and then determining the dry substance in each fraction separately, a moisture value of 11.56 to 11.60 per cent. was obtained (compare value without fractionation: 12.06 per cent.). These moisture values may be expected to be somewhat high since ether is generally considered to be unable to extract all the "fat" from maize. The ether extract of maize contains 6.5 to 7.4 per cent. of material which volatilizes *in vacuo* at 100° C. The results of this study unmistakably indicated that the major cause for the variability in the vacuum oven results can be attributed to the ether extract of the grain.

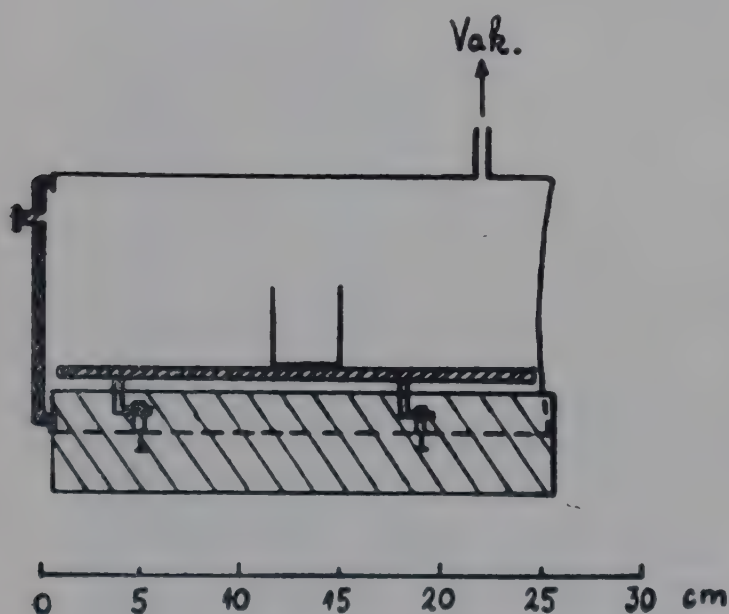


FIG. 1—Dich Vacuum-oven

The determination of the moisture content of the maize by the *reversibility procedure* yielded calculated moisture values ranging from 11.45 to 11.50 per cent., which are in close agreement with the values obtained by benzene or toluene distillation (11.51 per cent.), by the De Bruyn method (11.46 per cent.), and by the ether extraction method (11.58 per cent.). The results of this investigation lend strong support to the reversibility method which is based on the assumption that during drying, no change occurs in the primary water-combining properties of the material.

The final conclusion is that the true moisture value for this sample of 40-mesh maize lies within the narrow range of 11.46 to 11.55 per cent.

The results of this study would clearly indicate that the true moisture content of maize can be determined, that the moisture of the maize can be differentiated from "water of constitution," and that it is unnecessary to rely on empirical methods for its estimation. This conclusion is at variance with that obtained by all previous investigators in the moisture field, who state that it is impossible to determine the true moisture content of cereals and feeds. The moisture method has been considered to be purely empirical.

EXPERIMENTAL

Moisture Determinations in Barley and Malt by Drying in vacuo

The present experiments are built on the principle prescribed by Riiber for vacuum-drying of wort-extract and beer-extract in order to determine the true moisture contents under conditions so lenient that the extract is not destroyed. Riiber's experiments are so exact and reliable that no better foundation to work on is to be found, and at the same time it is a pleasure to introduce Riiber's work to modern cerealists; far too few chemists know this work which, unfortunately, was never published in the international reviews.

Plan of Investigation.

(1) By help of a Dich-vacuum oven, it is determined at which temperatures finely-ground barley and malt can be dried *in vacuo* over P_2O_5 with no other detectable alteration than loss of moisture.

(2) Under ideal vacuum drying conditions in the Riiber apparatus, it is then determined whether it is possible to dry to constant weight at these temperatures.

(3) Finally, certain reversibility studies have been made, in order to elucidate whether drying in the Riiber apparatus can be said to proceed reversibly.

Analytical Methods.—The construction of the Dich-vacuum oven is shown in Fig. 1. Briefly described, the oven consists of a horizontal cylindrical glass vessel, in the one end provided with a metal cover. By means of a level circular surface of contact and an

intermediary rubber disk the cover fits tightly to a corresponding level surface of contact on the glass. During the evacuation, the metal cover adheres to the glass. The cover is provided with an air valve. Built into the glass is an electric hot plate of metal with bimetallic temperature regulation, and

dish to be renewed at convenient intervals as often as necessary.

The *Riiber apparatus* is shown in Fig. 2. It consists of three parts: (1) the flask *A*, containing the desiccant P_2O_5 or H_2SO_4 . It serves as base for the whole apparatus; (2) the square-bent circulating tube *B*; and (3) the drying apparatus *C* itself, the construction of which is shown in Fig. 3. It consists of an inner glass tube *a* in the mantle *b*. The material to be dried is placed in the tube *a*. To the left, the tube *a* enters the circulating tube *B*, and to the right it is connected with the flask *A* through the widened tube *c* and the vertically descending tube *d*. The tube *c* is closed by a tight-fitting ground glass stopper *D*.

The glass mantle *b* is filled with glycerol and rests in an electric heater, the temperature of which is regulated automatically by the contact thermometer *T* and an electric relay. In this way it is possible in the tube *a* to keep a certain temperature with an exactness of $\pm 1^\circ C$. As appears from Fig. 2, the tube *d* passes through a cone connector into the flask *A* with neck joint ($\frac{3}{4}$ down the flask), and from the connector emerges

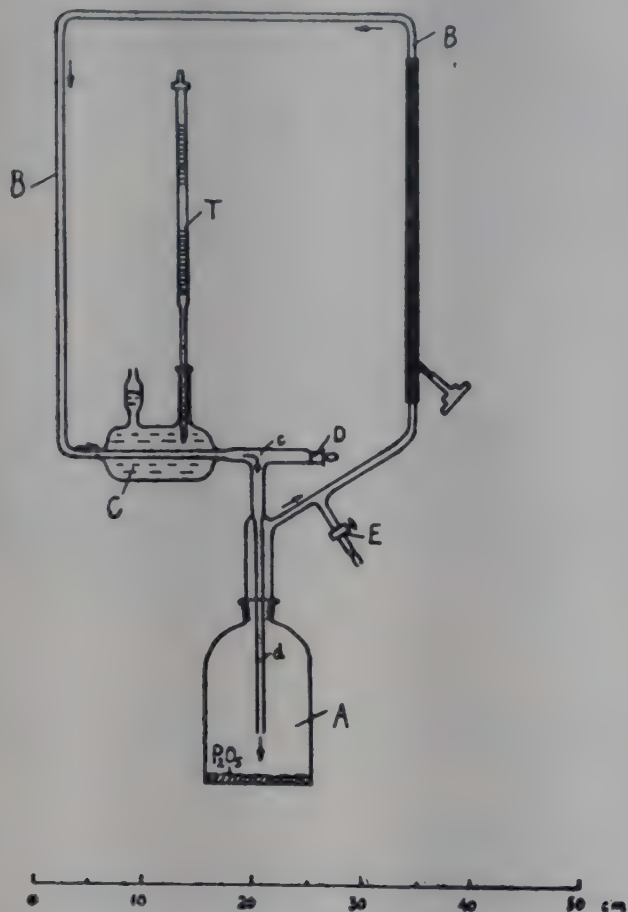


FIG. 2—Riiber Apparatus

the temperature can be regulated within $\pm 2^\circ C$. The glass is provided with a tube for evacuation by oil pump; complete vacuum can be maintained only by constant evacuation. The vacuum employed is approximately 5 mm. Hg. The temperature is controlled by a thermometer placed in a glass with barley meal or malt meal inside the oven.

For the drying, 2–5 gm. barley or malt meal is weighed into glass dishes; the covers are placed on edge during the drying process. After drying, air (dried through concentrated H_2SO_4 and twice through silica-gel) is used to release vacuum; the covers are put on and the dishes placed for 20 minutes in the desiccator with silica-gel before weighing. During the drying, P_2O_5 is placed in an open

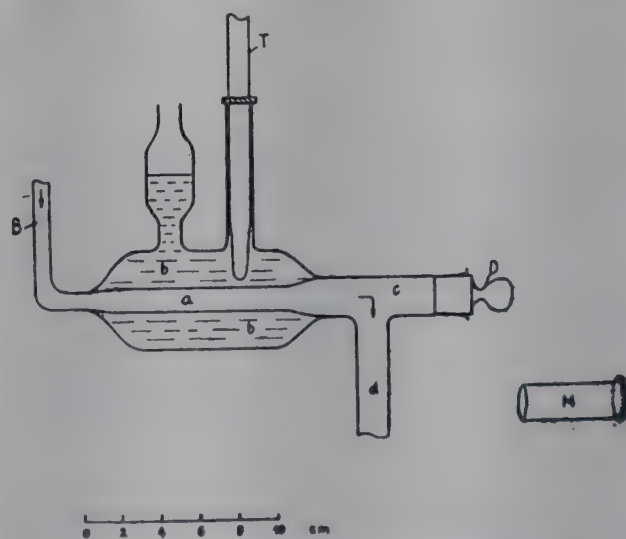


FIG. 3—Riiber Apparatus: drying apparatus, C.

the circulating tube *B* fitted with the stop-cock *E* for evacuation. The vertical branch of the circulating tube *B* (to the right) is enclosed in a copper tube which is heated by a gas flame. In this way the air in the closed apparatus is brought into a convenient slow circulation (*cf.* the arrows), thus letting only dried air pass through the tube *a*.

The substance to be dried is weighed into

a special glass tube, Fig. 4. It is open at both ends, allowing the circulating air to pass through. During cooling and weighing of the tube it can be closed by two tight-fitting glass mantles as appears from the figure. The weighing tube is approximately 9 cm. long and 1.2 cm. in outer diameter. The mantles being on, it weighs approximately 10 gm. Into the tube is weighed

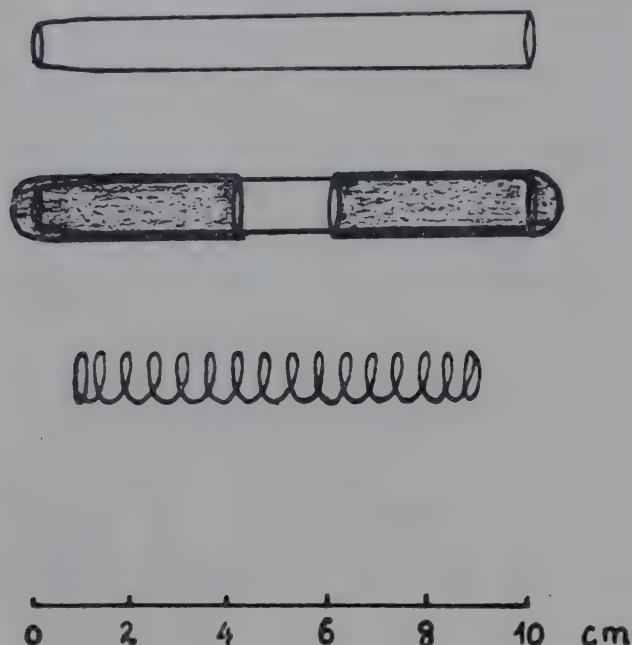


FIG. 4—Weighing Tube and Spiral for Rüber Apparatus

0.7–1.0 gm. of the substance to be dried. The weighing is carried out on a semi-microbalance, to be read off to five decimals.

The weighing tube is inserted in the following way: the stopper *D*, carefully greased with cock grease*, is taken away, and the protecting tube *M* (of metal) is put in in order to avoid the weighing tube being greased. By aid of a brass wire, the weighing tube is then pushed into the tube *a*, the tube *M* is removed, and the spiral *S* inserted; when the glass stopper *D* is again put in, the spiral will press the weighing tube entirely to the left in the tube *a* so that the circulating air can pass through the weighing tube. The drying finished, air is used to release vacuum, and this air passing the cock *E* has been dried through H_2SO_4 , and twice through silica-gel. If the glass stopper and joints are carefully greased, the apparatus is able to keep a vacuum unchanged for several

* Cock grease is made by melting together 9 gm. caoutchouc, 8 gm. paraffin and 32 gm. vaseline.

months. Furthermore, it will be seen that the apparatus allows variation of the drying conditions completely as desired. Temperature, vacuum, desiccant and gas in the apparatus can be varied at will.

Barley and malt is always ground to fine-meal on the Seck Mill (cone mill) according to the current Central European standard for brewery laboratories. This grinding is conventional and is not accompanied by characterization of fineness. After passing a Pfungstätter sieve 90 per cent. "meal" is required (i.e. fine sieve-fractions). To give non-brewing chemists an idea of the grinding, it can be said that it is very fine.

Concerning brewery malt-analyses in Central Europe (including Scandinavia) the moisture determination in malt is standardized by the Central European Committee of Analyses (the latest meeting of this committee took place in Munich in 1935). As standard is employed a drying in the Ulsch or V.L.B. apparatus for 3 hours at approximately 105°C . under normal atmospheric pressure. The Ulsch apparatus is the

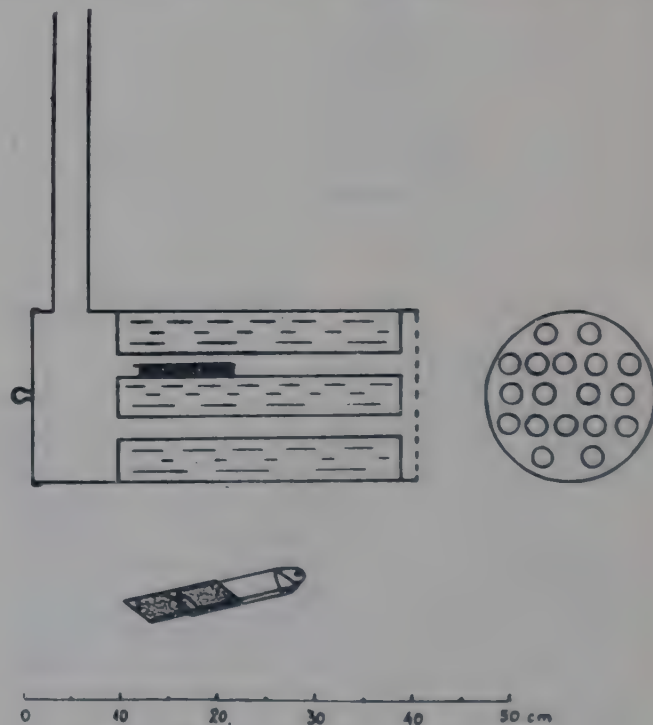


FIG. 5—Ulsch Apparatus

more widely known, and a short description of it may be given here. Its construction is shown in Fig. 5. It is a gas-heated water-filled tube boiler, in which water is made to boil under pressure slightly above atmospheric, corresponding to a temperature of between 100° and 110°C . The boiler has

10 to 30 tubes. In each of these can be placed a nickel boat with about 5 grm. of weighed substance. By the aid of the chimney, a natural draught of ordinary atmospheric air is provoked through the tubes. Owing to the construction of this apparatus it cannot be avoided that the moisture contents to be found are dependent on the height of barometer, on the degree of humidity of the air, on the degree of filling of the oven, and on the position of the tube in the oven. It is therefore difficult

to obtain satisfactory uniformity, even in the individual laboratory.
Determination of Suitably Mild Drying Temperatures by Drying in Dich's Oven in vacuo over P₂O₅.—Temperatures are required at which it is possible to dry barley and malt with no other detectable alteration than actual loss of moisture. In addition to Dich's oven some other ovens using somewhat different drying conditions (standard conditions) were employed, as appears from Tables II and III.

TABLE II
DRYING OF FINELY-GROUND BARLEY

Method.	Length of drying, showing period of essential constancy (hr.).	Final apparent moisture value (per cent.).	Colour after drying.
Ulsch: Air—104° C.—3 hr. 	—	16.02	—
Electric oven: Nitrogen, 1 atm.—93° C.—20 hr. 	—	16.39	—
Dich vacuum oven (° C.):			
105 	6- 48*	16.78	Slightly dark.
75 	10- 48*	16.58	Slightly dark.
52 	48- 96	16.50	No change.
40 	58-100	16.51	No change.

* Complete constancy not obtained.

TABLE III
DRYING OF FINELY-GROUND PILSENER MALT
(The Dich oven employed vacuum—P₂O₅ and the temperature shown)

Method.	Length of drying, showing period of essential constancy (hr.).	Final apparent moisture value (per cent.).	Colour after drying.
Ulsch: Air—105° C.—3 hr. (average of 18 determinations) 	—	3.87 (3.68-4.02)	Dark.
Air—105° C.—6 hr. (average of 18 determinations) 	—	3.89 (3.81-3.95)	Dark.
Electric oven: Air—100° C.—3 hr. 	—	4.03	Dark.
Nitrogen, 1 atm.—93° C.—20 hr. 	—	3.92	Dark.
Dich vacuum oven (° C.):			
100 	24- 96*	4.96	Dark.
80 	24- 96*	4.42	Dark.
61 	24- 72†	4.25	Slightly dark.
55 	48-120	4.12	No change.
50 	48-120	4.14	No change.
48 	72-120	4.12	No change.
43 	72-120	4.13	No change.
35 	96-144	4.12	No change.

* Constancy not obtained.

† Complete constancy not obtained.

To give an idea of the constancy attained by drying, two examples at 50° and 55° C. are shown (Table IV).

TABLE IV
DRYING OF MALT IN DICH'S VACUUM OVEN
Vacuum— P_2O_5
[Apparent moisture values (per cent.)]

Time (hour).	At 55° C.	At 50° C.
5	3.38	3.39
24	4.04	4.16
29	4.14	4.17
48	4.29	4.28
72	4.31	4.31
96	4.31	4.30
120	4.31	4.30

From these tables it appears that barley and malt can be dried to constancy within a period of about five days *in vacuo* over P_2O_5 at temperatures below 55° C.; also that barley is not quite so sensitive as malt to temperatures near 100° C.; further, that the moisture values found at temperatures of up to 55° C. *in vacuo* are somewhat higher than the moisture contents found in the Ulsch apparatus (air, 104° C.; 3 hr.) and in the electric oven (nitrogen, 1 atm., 93° C.; 20 hr.).

The purely subjective judgement of the change in colour of the meal during the drying gives but an indication, but still it shows that at temperatures above 55° C. a slight colouring of the substance takes place. Such a change of the material is so severe that drying at these temperatures can never comply with the stipulation that the drying must not be accompanied by any other detectable alterations than mere loss of moisture.

So attention should be drawn to the problem, whether other detectable alterations than loss of moisture take place at temperatures below 55° C. For a brewing chemist it is possible to employ malt for such an examination. Malt can be mashed, and both the mashing and the wort produced are to be estimated from the point of view of alterations occurring during the drying, whether of chemical nature (the different organic matters) or of enzyme character. A similar valuation cannot be formed as regards barley; here it is necessary to proceed differentially, estimating each type of organic

matter and each enzyme separately, but then the judgement is widely extended and at present it could hardly be thoroughly completed. In general, a thorough differential assessment will provide a more reliable knowledge than an integral one. Seen from a practical point of view the results from the integral method can be assumed to conform with the clause of the moisture definition "detectable alteration of any real significance," wherefore it appeared safe for the present to build on this function.

Malts from various dryings were mashed according to the so-called Congress method:

50 grm. malt (fine-meal) is mashed with 200 ml. water at 45° C. By constant stirring this temperature is maintained for 30 minutes; during 25 minutes the temperature is then raised by 1° per minute to 70° C.; 100 ml. water at 70° C. is added, and the mashing is continued for 60 minutes at 70° C. After cooling down to 20° C. the mash is diluted to 450 grm. and filtered through a folded filter.

During mashing the time of saccharification (*i.e.*, the iodine-normal time) at 70° C. was determined. In the filtrate the appearance, colour and extract content were determined. In some cases also the diastatic power of the malt was determined. Results appear in Tables V and VI.

The results of the mashings and the D.P.-determination show quite plainly that a drying to constancy *in vacuo* over P_2O_5 —without being accompanied by any other detectable alteration of any significance than loss of moisture—is possible in the temperature-interval 35°–45° C.; from 48° C. up to about 55° C. it is possible to observe a slight alteration, and in the interval from 60°–80° C. a stronger alteration of the dried malt. The alterations demonstrated are to be understood—as previously mentioned—as an integral function of the total number of alterations taking place during the drying.

Drying Under Ideal Conditions in the Riiber apparatus.—After this settling of suitable drying temperatures, the problem "drying to constancy" received closer examination.

Riiber's apparatus was chosen, since it offered such suitable conditions for complete drying. Barley and malt were employed, and the results are shown in Tables VII and VIII. In view of the exactness required

TABLE V
MASHINGS OF DRIED MALT-MEAL

Congress-mashing; for comparison with undried malt-meal the latter was weighed in a quantity exactly corresponding to 50 gm. dry matter. The Dich oven employed vacuum—P₂O₅ and the temperature shown.

	Time of sacchari- fication (min.).	Appearance of the wort.	Colour mm. (Stammer colorimeter).	Extract content of wort; Plato (per cent.).
Undried malt (moisture 4.1 per cent.) ..	15-20	Clear	32	9.07
Dried malt: Ulsch:				
Air—105° C.—3 hr.	35-40	Turbid	22	8.99
Air—105° C.—6 hr.	> 60	Turbid	18	8.97
Electric oven:				
Air—105° C.—3 hr.	> 60	Turbid	16	8.94
N ₂ —93° C.—20 hr.	> 60	Turbid	17	8.91
Dich vacuum oven (° C.):				
100—24 hr.	> 60	Turbid	16	8.94
80—24 hr.	> 60	Turbid	24	8.97
61—72 hr.	15-20	Turbid	27	9.01
55—120 hr.	15-20	Clear	29	9.10
50—48 hr.	15-20	Clear	31	9.04
50—120 hr.	15-20	Slightly turbid	30	9.04
48—48 hr.	15-20	Clear	32	9.06
43—48 hr.	15-20	Clear	34	9.08
43—120 hr.	15-20	Clear	33	9.07
35—144 hr.	15-20	Clear	32	9.06
Undried malt (moisture 4.3 per cent.) ..	15-20	Clear	32	9.13
Dried malt: Dich vacuum oven (° C.):				
55—120 hr.	15-20	Slightly turbid	30	9.10
50—120 hr.	15-20	Slightly turbid	31	9.11
43—120 hr.	15-20	Clear	32	9.14

TABLE VI
DIASTATIC POWER IN MALT AFTER DRYING
The determination was made according to Windisch-Kolbach, in accordance with the usual prescription for examination of malt. The total effect of α- and β-amylase on soluble starch at 20° C. is measured by this method.

	Diastatic power W-K-units per 100 gm. of malt dry matter.
Undried malt	135
Dried malt: Dich vacuum oven (° C.)—	
55	123
50	130
43	137
35	133

and semi-microbalance being employed, it was necessary to reduce all weighings to vacuum by correction for the buoyancy of the air. This was done by means of the following formula:—

$$p_{vac} = p_{air} + p_{air} \cdot d_{air} \left(\frac{1}{d_{glass}} - \frac{1}{d_{brass}} \right),$$

p indicating the weighed object, d the specific gravity. Brass weights and d_{glass} = 2.5 give the following simplified formula:—

$$p_{vac} = p_{air} + p_{air} \cdot d_{air} \cdot 0.28$$

The standard deviation of a single weighing was determined by repeated weighings of the same glass tube with intervals of one day. The glass tube was weighed in all 13 times, whereupon the deviation was found to be

$$(\Delta p_{vac})_{13} = 0.00003$$

The moisture content v appears from the following formula:—

$$v = \frac{p_{\text{moist}} - p_{\text{dry}}}{p_{\text{moist}} - p_{\text{empty}}} \cdot 100$$

$$= f(p_{\text{moist}} \cdot p_{\text{dry}} \cdot p_{\text{empty}}),$$

where $\Delta p_{\text{moist}} = \Delta p_{\text{dry}} = \Delta p_{\text{empty}} = 0.00003$
and

$p_{\text{moist}} = 12$ grm., $p_{\text{dry}} = \text{about } 11.9$ grm.,
and $p_{\text{empty}} = 11$ grm.,

wherefore the deviation in the moisture content, Δv , can be calculated from the following formula:—

$$\Delta v = \frac{1}{\sqrt{3}} \left(\frac{dv}{dp_{\text{moist}}} \cdot \Delta p_{\text{moist}} + \frac{dv}{dp_{\text{dry}}} \cdot \Delta p_{\text{dry}} + \frac{dv}{dp_{\text{empty}}} \cdot \Delta p_{\text{empty}} \right),$$

from which is found that

$\Delta v = 0.0035$ (the total range of deviation).

This means that the moisture content for a weighing of 1 grm. can be determined within ± 0.002 per cent. absolute.

It is thus justified to include three decimals in the results.

From these tables will be seen for malt, that by continued drying at 49°C . a decomposition of the malt takes place, corresponding to a loss of weight of about 0.01 per cent. absolute per seven days; this decomposition, however, does not seem to begin until after about 14 days. So it will be possible for about 14 days to undertake a drying practically to constancy, following the Riiber principle of weighing after 7 and 14 days respectively. Also the decomposition at 40°C . is considerably smaller, amounting only to a loss of weight of about 0.002 per cent. absolute per 10 days. A drying to constancy at 40°C . will therefore, from a theoretical point of view, be essentially safer than at 49°C . The difference, however, is not big enough to assert itself in practice, if the drying period does not exceed about 14 days. Again, the corresponding moisture determinations from Dich's vacuum oven are well in conformity with the Riiber values.

For barley, by continued drying at

TABLE VII
VACUUM-DRYING OF MALT IN THE RIIBER APPARATUS
(Apparent moisture values per cent.; drying agent P_2O_5)

Vacuum (mm. Hg) Temperature ($^\circ \text{C}$.)	12 49	9 49	6 49	6 49	6 49	8 49	8 40	8 40
Drying period (days)								
1	4.443	—	—	—	—	—	—	—
2	—	4.411	—	—	—	—	—	—
3	4.545	4.416	—	—	—	—	—	—
4	4.591	4.433	—	—	—	—	—	—
5	—	4.444	—	—	—	—	—	—
6	4.619	4.444	—	—	—	—	—	—
7	4.617	4.438	4.599	5.168	4.888	5.248	4.602	4.438
9	—	4.448	—	—	—	—	—	—
10	4.622	—	—	—	—	—	—	—
12	—	4.451	—	—	—	—	—	—
14	—	—	4.604	5.175	4.885	5.246	4.606	4.443
16	—	4.457	—	—	—	—	—	—
23	—	4.514	—	—	—	—	—	—
41	—	—	4.635	5.190	4.910	5.290	4.610	4.450
69	—	—	4.731	5.272	5.102	5.338	—	—
81	—	—	—	—	—	5.353	—	—
100	—	—	—	—	—	5.370	4.621	4.458
165	—	—	—	—	—	5.415	4.633	4.470
Drying in the Dich vacuum oven to constancy, 4 days, at corresponding temperatures ..	4.61	4.43	4.59	5.18	4.87	5.25	4.63	4.41

TABLE VIII
VACUUM-DRYING OF BARLEY IN RIIBER APPARATUS
(Apparent moisture value *per cent.*; drying agent P_2O_5)

Vacuum (mm. Hg) ..	10	9	11	11
Temperature ($^{\circ}$ C.) ..	49	49	40	40
Drying period (days)				
7	13.232	12.897	13.353	13.670
14	13.241	12.902	13.352	13.681
41	13.244	12.908	13.348	13.679
56	13.242	12.910	13.352	13.685
71	13.248	12.912	13.355	13.690
Drying in the Dich vacuum oven to constancy, 4 days, at corresponding temperatures	13.23	12.92	13.32	13.67

40° – 49° C. a slight decomposition takes place, corresponding to a loss of weight of about 0.003 *per cent.* absolute *per* 10 days. Thus barley is not quite so sensitive as malt for drying at 49° C. It will therefore be possible in about 14 days to undertake a drying practically to constancy, following the Riiber principle. Moisture determinations from Dich's vacuum oven are well in conformity with the Riiber values.

With only one Riiber apparatus available, the investigations took much time, and it was possible only to investigate the drying process by relatively few examples within a limited interval of temperature. This interval was chosen on the basis of the previous results of the vacuum dryings in the Dich vacuum oven, wherefore these results were able to give information of practical value to elucidate the problem of "drying to constancy."

Reversibility Studies.—The problem of reversibility was examined in the Riiber apparatus by alternate drying to constancy over H_2SO_4 and P_2O_5 . H_2SO_4 being a less powerful desiccant than P_2O_5 , a sample dried over H_2SO_4 will still contain a small quantity of moisture. A sample dried over P_2O_5 , on the contrary, can be supposed to be perfectly dry. If the moisture that is lost on transfer of the sample from storage over H_2SO_4 to storage over P_2O_5 is completely regained on transfer of the sample back to the same H_2SO_4 storage, it is to be supposed that the complete drying over P_2O_5 was not accompanied by any decomposition: (It is most essential that the H_2SO_4 reservoir is exactly the same the

first and second times; therefore, the flask with H_2SO_4 is kept safely closed with a ground-glass stopper during the drying over P_2O_5 .)

In order to obtain definite conclusions, these reversibility-studies must be carried out in such a way that the small decomposition of barley and malt, which in spite of all takes place, cannot significantly assert itself. In accordance with the previous dryings in the Riiber apparatus this will be possible by employing a drying temperature of 40° C. and a drying period not exceeding 14 days. The results of these reversibility dryings are gathered in Table IX.

It will be seen that the moisture lost on transfer of the sample from storage over H_2SO_4 to storage over P_2O_5 is completely regained on transfer of the sample back to the same H_2SO_4 storage. Owing to the conditions under which the drying proceeds, the inevitable small decomposition cannot be observed within the drying intervals applied, wherefore it is to be concluded that the drying of barley and malt *in vacuo* over P_2O_5 at 40° C. proceeds approximately reversibly. Any "important" decomposition during the drying would render a reversible moisture absorption after complete drying unlikely.

The careful and decisive work of Sair and Fetzer, which became available at this stage, made it clear that further investigations on these lines could provisionally stop, and a thorough investigation of the method of distillation (which had been planned) now seemed quite needless. It seemed much more important to develop the above-mentioned

TABLE IX
REVERSIBILITY DRYINGS OF BARLEY AND MALT IN THE RIIBER APPARATUS WITH ALTERNATE
DRYING OVER H_2SO_4 AND P_2O_5 *IN VACUO*
(Apparent moisture value *per cent.*)

	Malt.		Barley.	
	11 mm. Hg H_2SO_4 40° C.	12 mm. Hg H_2SO_4 40° C.	13 mm. Hg H_2SO_4 40° C.	10 mm. Hg H_2SO_4 40° C.
7 days ..	4.878	5.165	12.802	13.242
14 days ..	4.870	5.171	12.809	13.242
	12 mm. Hg P_2O_5 40° C.	12 mm. Hg P_2O_5 40° C.	12 mm. Hg P_2O_5 40° C.	9 mm. Hg P_2O_5 40° C.
7 days ..	4.987	5.342	12.989	13.427
14 days ..	4.991	5.348	12.987	13.430
	10 mm. Hg H_2SO_4 40° C.	10 mm. Hg H_2SO_4 40° C.	12 mm. Hg H_2SO_4 40° C.	10 mm. Hg H_2SO_4 40° C.
7 days ..	4.875	5.178	12.814	13.252
14 days ..	4.880	5.172	12.811	13.247

work into a connected whole, to form the basis for common lines in moisture determinations. It appeared that the time was ripe for such a step, considering both the valuable investigations made during recent years and the desire of avoiding the variety of standardized moisture determinations, now in force within cereal chemistry.

It seemed both natural and correct to introduce this material to the Fourth Scandinavian Meeting of Brewing Chemists in Oslo in May, 1948. Brewing chemistry is a branch of cereal chemistry, for which moisture determinations play an important rôle. Also the Scandinavian Meetings of Brewing Chemists have the necessary power and reputation to be able to proceed with the suggested proposal for standardizing the principle for moisture determination.

Reference Method for Moisture Determinations

Based upon the definition of moisture, given in the Introduction, it is now possible, with the help of the above-mentioned experimental results to formulate a reference method as close to the definition as can be allowed by the present stage of development of knowledge and technique. As reference method the following is suggested:—

Generally.—Drying to practically constant weight of the finely-ground substance *in*

vacuo (from 1 to 10 mm. Hg) over P_2O_5 at about 40° C. The following vacuum ovens are to be recommended for this purpose:—

- De Bruyn's vacuum apparatus. This apparatus is extremely well fitted for reference apparatus, being very simple and allowing highly reliable determinations.
- Riiber's vacuum apparatus. This apparatus also allows very reliable determinations, but may be too complicated to obtain general acceptance.
- Any vacuum oven allowing the fulfilment of the given conditions. As a Danish example, Dich's vacuum oven may be mentioned.

For special Cases.—For drying of particularly moist substances, or for drying of substances whose content of volatiles is somewhat higher, it is necessary to ensure that the vacuum drying over P_2O_5 at 40° C. is in conformity with the results of reversibility investigations. For such cases, Sair and Fetzer's reversibility method is particularly to be recommended, owing to its being built on a correct principle.

That the reference method for cereals and malt is in conformity with the moisture definition appears from the following:—

(i) *Drying is Reversible.*—This is proved by Sair and Fetzer's reversibility studies (*cf.* above), and also by the present reversibility studies concerning barley and malt, for which studies the Riiber apparatus was employed with alternate drying over H_2SO_4 and P_2O_5 .

(ii) *Drying to Practical Constancy is Possible.*—This is proved by Sair and Fetzer's vacuum dryings at 38° – 50° C. in the De Bruyn apparatus (*cf.* Table I). Here constancy of weight was found from the 27th to the 40th day both by drying maize alone and by drying maize treated with alcohol to increase the surface; it is also proved by Sair and Fetzer's fractional drying of the ether-soluble and the ether-insoluble constituents (Table I). It is further proved by the present vacuum dryings of barley and malt in the Riiber apparatus at 35° – 50° C., following the Riiber principle for drying to constancy (repeated weighing after the lapse of a period of the same duration as that before the first weighing). Absolute constancy of weight is not obtainable; malt will lose approximately 0.01 *per cent.* absolute *per week* by constant drying at 50° C.; at 40° C. the loss is only 0.002 *per cent.* absolute *per 10 days*; barley will lose approximately 0.003 *per cent.* absolute *per 10 days* in the temperature interval 40° – 50° C. These values were found by drying for up to 165 days, and decomposition is, unlike evaporation, fairly proportional with time. *Practical constancy of weight*, on the other hand, is possible in the temperature interval 40° – 50° C., if the drying is not continued for more than about 14 days. The decomposition is too small to make itself perceptible. So drying to practical constancy of weight is easily obtainable in Dich's vacuum oven. (It should be added that Riiber formerly found a somewhat greater decomposition of beer-extract and wort-extract, corresponding to 0.1 *per cent.* absolute *per week*; this drying, however, was performed at 55° C. over H_2SO_4 . The mere difference of temperature can hardly explain the comparatively greater decomposition, but experience suggests that drying over H_2SO_4 is accompanied by a greater decomposition than over P_2O_5 , probably as a consequence of the air being saturated with H_2SO_4 . 95 *per cent.* H_2SO_4 will at 35° C. have a vapour pressure of 0.015 mm. Hg; the total vapour pressure will

approximate to the partial pressure of the water, and so the partial pressure of the sulphuric acid will be but a small part of the total, but it has not been possible to find an exact indication. It is a matter of experience that drying over H_2SO_4 gives a sulphuric acid "film" on the inside of the apparatus, while a drying over P_2O_5 leaves no such film.)

Finally, it is to be mentioned that Berglund—by drying barley at room temperature over P_2O_5 —found a constancy of weight from the 34th till the 60th day. Even if nothing can be concluded as regards constancy of weight at 40° C., this is still an interesting experimental fact.

(iii) *The Drying does not bring about other Detectable Alterations of any Significance than the Reversible Loss of Moisture.*—This is proved by the present study of dried malt, by which investigations the contingent alterations of the malt were examined as integral functions of the total mashing properties of the malt; it was found that drying at a temperature of up to about 45° C. takes place without detectable alterations of the properties of the malt.

After all, the notion of "alterations of any significance" is as yet subject to a certain degree of personal judgment, and this point must in future be placed in the front line of the endeavours of cereal chemists in order to support the conditions of the reference method on reliable, theoretical knowledge. The fact that mashing properties were chosen as indicator does not mean that this choice is thought absolutely correct and exhaustive; it is but a step on the way to perfectly reliable knowledge.

When laid down as working definition, however, the moisture definition must, to retain validity in future, contain the above-mentioned alterations as a condition. As long as our knowledge is imperfect, it is easily imaginable and therefore allowable that inevitable alterations occur, which are unrelated to moisture and not associated with any loss of weight. Such alterations need not necessarily limit the conditions under which the "reversible" drying can freely proceed. An illustrative example is given by Berglund in his reference to literature concerning the partial transformation of the starch into soluble starch after drying *in vacuo* at 25° C. for five weeks. As long

as it is not known wherein the transformation starch $\rightarrow$ soluble starch consists it is permissible to class it among such alterations that in justice are estimated as being of no importance. Not until it is proved directly, that the alteration involves moisture, or is connected with a loss of weight, can it in justice be regarded as being important.

(iv) *The Drying is to be Considered as Complete.*—This is made probable by Sair and Fetzer's investigations. By direct moisture determinations by distillation with relatively low-boiling immiscible solvents, benzene and toluene, complete conformity was found with the De Bruyn vacuum determinations, with the fractional drying of ether-soluble and ether-insoluble constituents and with the calculated moisture contents based on reversibility studies (Table I).

The reference method is to be considered as a time-limited practical execution of the given moisture definition. It is experimentally secured by the relevant experimental facts valid at a particular time, and the speediest possible drying is chosen, i.e., practically the highest temperature and the greatest vacuum permissible, to reach the final aim of the moisture determination, as was laid down in the introduction.

In this connection it should be added that Sair and Fetzer seem inclined to attach some theoretical importance to their direct moisture determinations by distillation with benzene and toluene; they are not far from ascribing chief importance to the distillation results with standardization in view, but they do not go so far. It is very valuable to know that the distillation method is giving perfectly correct results, but it would be wrong in principle to standardize such a method. The method affords no practical possibility of assessing which moisture is being determined, and is therefore

theoretically insecure. It belongs to the practical methods (cf. the Introduction), but differs so widely from ordinary biochemical working methods, that it should not be attempted to standardize it for problems of a biochemical nature. The validity of the principle of the method could not be assumed in future.

As regards daily methods employed in individual laboratories the following is valid: with every attention to the exactness and speed required, any method can be adjusted to the reference method. It is assumed that the latter can be executed everywhere. Thus there is a free choice of drying methods, distillation methods, electrical methods, chemical methods, etc. Then the many standard conditions for these methods would be omitted, because in future they could all be referred to the reference method.

REFERENCES

1. V. Berglund, *Danske Møller*, 1936, 9, No. 1, 3; No. 2, 2.
 2. G. L. Bidwell and W. F. Sterling, *Industr. Engng. Chem.*, 1925, 17, 147.
 3. L. de Bruyn and F. H. van Laent, *Rec. Trav. chim. Pays-Bas*, 1894, 13, 218.
 4. J. E. Cleland and W. R. Fetzer, *Industr. Engng. Chem., Anal. Ed.*, 1941, 13, 858; 1942, 14, 27, 124.
 5. J. S. Ford and J. M. Guthrie, *this Journ.*, 1905, 326.
 6. G. Malfitano and A. Moschkoff, *C.R. Acad. Sci., Paris*, 1912, 154, 443.
 7. L. M. Pidgeon and O. Maass, *J. Amer. chem. Soc.*, 1930, 52, 1053.
 8. C. N. Riiber, *Z. ges. Brauw.*, 1890, 13, 97.
 9. C. N. Riiber, *Z. ges. Brauw.*, 1891, 14, 547.
 10. C. N. Riiber, *Norsk Videnskabselskabs Skrifter, Christiania*, 1897, 82 pp.
 11. L. Sair and W. R. Fetzer, *Cereal Chem.*, 1942, 19, 633.
 12. A. R. Urquhart and N. Eckersall, *J. Text. Inst., Manchr.*, 1930, 21, 499r.
- 12th July, 1949.

BREWING TRIAL WITH A NEW VARIETY HOP

Ref. No. OT48

By L. FLETCHER, F.R.I.C.

Small-scale brewing trials with this variety, which has previously been found to have some degree of resistance to *Verticillium* wilt, have shown that this hop imparts no objectionable flavours to beer and it can therefore be strongly recommended for future trials.

THIS hop was raised in 1927 by Professor Salmon at Wye College and was selected for small-scale brewing trial from a number of samples grown in 1947, firstly, because it is a hop of pleasing appearance and aroma, and also it was said to have some degree of resistance to *Verticillium* wilt. The latter statement has since been corroborated, for recently Dr. W. G. Keyworth has privately reported "that from tests made in 1947 and 1948 the variety OT48 may be classified as moderately resistant to *Verticillium* wilt,

Physical Appearance.—A good looking hop with a rich side. The cones are well shaped, smallish and berry-like. Aroma very pleasant but not strong.

Brewing Trials.—These were carried out in the laboratory, using Pale Ale all malt wort of O.G. 1048. Methods of boiling, etc., were as employed previously (this *Journ.*, 1944, 80). The amount of copper hops taken was in the inverse ratio of the percentage α -acid content of the natural hop calculated on a standard hop of 5 per cent. α -acid. Thus,

ANALYSIS.

Year of growth.	Variety.	Date of analysis.	Per cent. on dry hop.		P.V. = 10 α .
			α -acid.	β -resin.	
1947	OT48	17/3/48	6.52	8.88	65
1947	OT48*	20/2/49	4.62	8.82	46
1947	OT48	29/3/49	3.55	—	36
1948	OT48	27/12/48	5.68	9.15	57
1947	Fuggles. J. Day	2/8/48	4.95	—	50
1947	Goldings. Rickard and Son	1/10/48	5.50	—	55
1947	Goldings. Redsell	15/2/49	5.00	—	50

* The same pocket was used; this was kept in cold store at 38° F.

i.e., similar to the varieties OR55 and OJ47. It is considered to be one of the most promising of the newer selections and may prove of value for replanting infected gardens after a fallow period. Further tests are in progress."

In view of this favourable report from Dr. Keyworth, these small-scale brewing trials and dry hopping tests assume a greater significance and they should encourage the further development of this variety. Another point to be noted from the analytical figures is that OT48 shows promise of being a reasonably good keeper.

Parentage.—Bramling crossed with a male hop seedling (Ref. No. OL45) raised from the "open" flowers of a wild hop from Manitoba, Canada (Ref. No. BB1) growing at Wye College.

in the table, the normal hop rate is given with the actual rate in parenthesis.

Dry Hop Test (5/10/48). Laboratory scale. Four oz. *per* barrel in commercial Pale Ale.

OT48 (1947).—After six weeks. Flavour very good and not over bitter.

Goldings (1947).—After six weeks. Flavour very good and not over bitter.

After a further two weeks in bottle both beers had a good round flavour and pleasant bitter. Beers were very similar in flavour.

Dry Hop Test (18/2/49).—This test was carried out in 18-gallon casks in the brewery, using special Pale Ale dry-hopped at rate of 3 oz. *per* barrel. Both 1947 and 1948 growths of OT48 were tested, using 1947 Redsell Goldings as a control.

TRIAL 4/8/48. (Control = J. Day, Fuggles.)

No.	Hop.	Oz. per barrel.	Age of beer in weeks when tasted.	Copper hop test.
1	OT48 alone (1947)	16 (14.5)	2	Pleasant—mild beer.
			3	Good. A pleasant hop flavour.
2	OT48 alone (1947)	20 (18.2)	2	Pleasant flavour. A mild hop bitter.
			3	do. do.
3	OT48 } blend Fuggles }	20 (3.6 + 13.4)	2	Satisfactory blend. Very pleasant.
			3	do. do.
4	Fuggles alone	16 (17.8)	2	Very good beer, mild.
			3	More bitter, but excellent beer.
5	Fuggles alone	20 (22.3)	2	Good, but a stronger bitter.
			3	Quite good, but rather bitter.

TRIAL 4/10/48. (Control = Rickard and Sons, Goldings.)

No.	Hop.	Oz. per barrel.	Age of beer in weeks when tasted.	Copper hop test.
6	OT48 alone (1947)	16 (14.5)	3	Very good mild bitter beer.
			6	Most pleasant bitter.
7	OT48 alone (1947)	20 (18.2)	3	Very good, pleasant flavour.
			6	Very good hop flavour and bitter.
8	OT48 } blend Goldings }	20 (4.6 + 15.0)	3	Fair blend—pleasant.
			6	Blend good, but not equal to hop alone.
9	Goldings alone	20	3	Too bitter.
			6	Still too bitter, not unpleasant.

The casks were sampled at two, three and four weeks and the examination was carried out "blind" by a number of tasters, independently. Their opinions were unanimous in that the differences between the three beers were scarcely detectable and that in every case the beers were of excellent flavour and of good hop character without being pronounced. The value of further trials with this promising variety is indicated.

The author thanks the Directors of Messrs. Wm. Younger and Co., Ltd., for permission to publish these results, and his colleagues, Messrs. R. P. McLintock and G. A. Downie, for their assistance in carrying out the brewing trials.

Abbey and Holyrood Breweries,
Edinburgh.

May, 1949.

BREWING OF KAFFIR BEER IN NORTHERN RHODESIA

By R. S. YOUNG, Ph.D., F.R.I.C.

(Central Laboratory, Nkana, Northern Rhodesia)

In a typical process, the cereals used are Kaffir corn (*Sorghum* spp.), millet (*Eleusine coracana*), or bulrush millet (*Pennisetum typhoides*). A mash, made from moistened and stored grain, is treated with a "yeast mixture" to give a product containing 3-3.5 per cent. of alcohol and ~20 per cent. of suspended solids, providing a valuable source of vitamins and salts.

ONE of the many problems which the large mining companies had to face when they commenced operations in Northern Rhodesia was the provision of kaffir beer for their native workers and their families. Kaffir beer, a grey, yeasty liquid resembling a very thin gruel, has long been an important item in the life of most Central African natives, and under village conditions a portion of the cereal crops is usually laid aside for the production of beer. In the mine compounds, however, where food is provided for the native workers and their families, it was obvious that a small brewery would have to be operated to cater for the needs of the many thousands of natives attached to each mine. At each town, therefore, a small brewery was established under the control of the local Township Management Board to produce kaffir beer, which is sold at a reasonable price in beer halls in the compounds. Profits from the sale of beer are allocated to native welfare organizations.

The following description of the brewing process at Nkana, the site of the great copper-cobalt operations of Rhokana Corporation, is typical of those in vogue in Northern Rhodesia. The cereals favoured for kaffir beer are kaffir corn, which may be one of several species of sorghum such as *Sorghum caffeorum*, *S. coriaceum*, or *S. conspicuum*, and millet, exemplified by finger millet, *Eleusine coracana*, or bulrush millet, *Pennisetum typhoides*. Both kaffir corn and millet are aboriginal African crops and grown well over most of the central part of that continent. A mixture of kaffir corn and millet is preferred by natives for their beer.

The kaffir corn and millet are moistened with water and kept damp under canvas in an open, thatched-roof building for three days. They are then spread out on a large concrete

slab in the sun until dry, this operation requiring 1-3 days, depending on atmospheric conditions. The corn and millet are then mixed in the ratio of 7 to 3, respectively, and ground in a hammer mill to approximately 10 mesh.

About 65 lb. of this mixed grain are measured into a 44-gallon steel drum and 15 gallons of hot water are added. After stirring, the drum is filled with cold water, again stirred, and the contents then transferred to cooling tanks.

When three drums, comprising 132 gallons of mash, have been emptied into the cooling tanks, 20 gallons of "yeast mixture" are added. The preparation of the latter will be described later. The mash and yeast mixture are allowed to remain together overnight and in the morning the liquid is strained through a metal screen approximating 14-mesh to remove the husks and coarser cereal particles. It is pumped to storage tanks and from these to a lorry having a steel tank holding 350 gallons for delivery to the beer halls of the compound. Here it is sold directly from storage drums, in metal mugs holding a pint, quart, or two quarts. The beer is consumed on the premises and none is sold in bottled form.

The yeast mixture is started with unsoaked kaffir corn only. To 200 lb. of the latter are added 45 gallons of hot water. When this is cool, usually overnight, it is mixed with 200 lb. of the ground mixture of kaffir corn and millet. A 44-gallon drum is two-thirds filled with hot water and boiled. To this water is added 10 gallons of the yeast mixture, and this is cooked for 8 hours, stirring at regular intervals. The contents are then put in cooling drums. When cool, the mixture is transferred to a 44-gallon drum up to the two-thirds mark and 60 lb. of soaked mixed meal, *i.e.* kaffir corn and

millet, are added. This mixture is allowed to stand one day in hot weather and two days in cold weather to ferment, and is then ready to add to the mash, as indicated above, in the ratio of 20 parts of yeast mixture to 132 parts of mash.

To technical men in large modern overseas breweries these operations must seem extremely crude. It must be remembered, however, that the entire operation is under the supervision of only one European, whose time is also occupied with oversight of deliveries and sales in the beer halls. On Sundays, consumption of beer may reach 1,400 gallons for the 20,000 adult natives of Nkana. Small wonder that brewing operations must be reduced to a minimum and procedures simplified to bring them within the capabilities of native employees.

The alcoholic content of kaffir beer cannot, by law, exceed, 4 *per cent.* by volume, and by this brewing procedure it usually ranges from 3 to 3.5 *per cent.* The suspended solids are in the neighbourhood of 20 *per cent.* These, not being subjected to cooking or discarding of their soluble salts, are a valuable source of vitamins and minerals for the native diet. It has been shown² that kaffir corn meal of Northern Rhodesia on the "as received" basis, is fairly high in Ca, P, Fe, and Cu. South African workers¹ have indicated that kaffir beer in that country contains as much vitamin B1 (thiamin), B2 (riboflavin), and nicotinic acid, as most fruits, and more vitamin C (ascorbic acid) than cereals.

The beer is sold in the native beer halls at the price of 3d. *per* pint. With the protein, carbohydrate, and fat content, together with the vitamin and mineral value, of the suspended solids in this beverage the food value of kaffir beer is considerably higher than that

of beer prepared for white races. From a nutritional and economic viewpoint the native undoubtedly receives good value for his money with this beverage.

Those concerned with the production of kaffir beer in Northern Rhodesia realize there is much scope for improvement. Progress has been retarded by the war and post-war shortage of materials and equipment designed to give a better control over the final product. Experiments along various lines in the brewing process have had to be deferred while the technical staffs of the mining companies were engaged on metal production. Methods have been evolved from experience only, and have been designed to suit local labour and the simple requirements of the consumers. Technical supervision is almost entirely lacking, except for periodic determinations of the alcohol content of the beer.

Kaffir beer, as produced in the mining towns of Northern Rhodesia, is infinitely more uniform and more hygienic than the beer made in native villages. It falls far short, however, of the usual beer produced for consumption by whites, by virtue of the lack of that close technical control over all processes which yields a uniformly good product. It must be the task of the chemical engineer, biochemist, mycologist, and practical brewmaster in Northern Rhodesia to put the production of kaffir beer on a proper scientific basis.

REFERENCES

1. F. W. Fox and L. Goldberg, *South African Institute for Medical Research*, 1944, 9, 123.
2. R. S. Young and A. Gollidge, *Emp. J. exp. Agric.*, 1947, 15, 89.

July, 1949.

JOINT ACTION OF α - AND β -AMYLASES

III. FURTHER OBSERVATIONS AND CONCLUSIONS

By I. A. PREECE, M.Sc., Ph.D., F.R.I.C., and M. SHADAKSHARASWAMY,
M.Sc., Ph.D., A.R.I.C.

Although at mashing temperature (e.g., 149° F.) β -amylase is active for no more than 15 minutes in laboratory starch conversions, its activity may persist for 40-60 minutes in malt extracts at this temperature. The activity of α -amylase, on the other hand, is lost more rapidly in malt extracts than in laboratory starch conversions, but its survival in extracts is enhanced by the use of malts of high D.P. The activity in a mash at a given time is profoundly influenced by the initial D.P. of the malt; also, with β : α ratios greater than 3½:1, the ratio is without influence on the production of fermentable reducing groups at a given D.P. level, but fermentable reducing group production increases with increase in D.P. Thus, a knowledge of D.P. is more generally useful than that of individual amylase contents. Indirect evidence suggests that the starch products in wort are glucose and maltose and straight- and branched-chain dextrans of mean molecular size equivalent to 7-8 glucose units, though the presence of trisaccharides and of a maltose isomer is not excluded; the susceptibility of the dextrans to amylase attack is discussed. It is emphasized that, in the present state of knowledge, great care must be exercised in attempting to apply the results of laboratory experiments to mash tun problems.

INTRODUCTION

A study is reported in the second paper of this series (this *Journ.*, 1949, 298) of the influences of the ratio of β : α -amylase concentrations, of total amylase concentrations, and of temperature on the liberation of reducing groups from starch. Thus, certain general principles have been established. With a total enzyme concentration relative to starch exceeding that which would be found in a brewery* malt of D.P. greater than 15, the ratio β : α has little influence on the observed results at a given temperature approximating to that of an infusion mash so long as this ratio exceeds about 3½:1, a ratio close to the average found for Scottish brewery malts (Preece, this *Journ.*, 1947, 154; 1948, 141). Again, to increase the temperature in the range investigated (63-67° C., 145-153° F.) is equivalent in respect of reducing group production to using either a lower total enzyme concentration or a lower ratio of β : α -amylases.

However, from a practical point of view, the proportion of reducing groups produced under a given set of conditions is of less significance than a knowledge of the extent to which the products are fermentable. Hence, to obtain fuller advantage from the

results already described, three further types of evidence are necessary, *viz.*, the influence of variations of enzyme ratios and concentrations and of temperature on the production of fermentable materials, the survival of amylases at mashing temperature in malt extracts, and the degree of starch conversion obtained when malt is mashed with water. The present report presents evidence directly on the two first of these problems, while an approximate indirect solution to the third is possible knowing the composition of an average malt.

EXPERIMENTAL

Starch Conversions and Fermentations.—1 per cent. soluble starch, buffered at p_H 5.4, was hydrolysed at 63°, 65° or 67° C. (145°, 149° or 153° F.) with mixtures of precipitated α - and β -amylases under the conditions previously described (Part II, *loc. cit.*); reference to the previous paper will provide full details of the arbitrary enzyme units employed, and of the D.P. equivalents of the various β : α ratios at the total enzyme concentrations used when acting on this substrate. In the present conversions, 200 ml. quantities were used, and at the end of the required periods of hydrolysis

TABLE I
MALTOSE "RECOVERY" BY FERMENTATION IN PRESENCE OF PRODUCTS OF STARCH
HYDROLYSIS BY α -AMYLASE

(Results expressed as mgrm. of apparent maltose)

	Before fermentation.		After fermentation.		Fermentable reducing groups.		Maltose added.		"Recovery" equals additional maltose fermented.	
	Fe.*	Cu.†	Fe.	Cu.	Fe.	Cu.	Fe.	Cu.	Fe.	Cu.
Conversion alone	310.0	257.0	250.6	182.5	59.4	74.5	—	—	—	—
With added maltose	672.3	625.0	248.9	185.5	423.2	439.5	362.3	368.0	363.8	365.0
Conversion alone	283.0	237.0	229.6	173.1	53.4	63.9	—	—	—	—
With added maltose	607.0	555.0	231.0	172.3	376.0	382.7	324.0	318.0	322.6	318.8
Conversion alone	305.2	257.0	248.3	184.8	56.9	72.2	—	—	—	—
With added maltose	616.0	568.0	246.1	187.0	369.9	381.0	310.8	311.0	313.0	308.8

* Ferricyanide method.

† Fehling's titration.

the enzymes were inactivated by boiling the conversions for 1 minute. After cooling, 4 ml. of sterile yeast water (5 gm. of pressed yeast *per* 100 ml. of yeast water) were added to each conversion and the volumes returned to 200 ml. Reducing group determination was carried out both with ferricyanide and by Fehling's method.

For each fermentation, 100 ml. of hydrolysate in a sterile flask was mixed with 2 gm. of pressed brewery yeast which had been washed immediately beforehand at the centrifuge with 100 ml. of distilled water. After thorough shaking, the flask was incubated at 21° C. (70° F.) for 48 hours. The contents of the flask were then transferred to a 150-ml. graduated flask and made up to the mark. The suspension was centrifuged to cause separation of the yeast, and reducing group determinations were carried out on suitable aliquots of the clear supernatant liquor, using ferricyanide in all cases and Fehling's titration wherever the concentration of reducing substances allowed. Fermentable reducing groups are obtainable by difference, and the results under the different conditions of experiment are shown in Tables II and III.

The reliability of the method for determining maltose added to α -amylase conversions is shown in Table I. In obtaining the results there shown, three independent portions of 1 *per cent.* soluble starch solution at p_H 5.4 were hydrolysed for 15 minutes at 65° C. (149° F.) with an appropriate addition

of α -amylase. After enzyme inactivation and volume adjustment, reducing groups were determined and parallel fermentations were carried out on portions of liquor which were identical except that crystalline maltose had been added to one of each pair. It will be observed that maltose "recovery" ("additional maltose fermented") is 99.6–100.7 *per cent.* by the ferricyanide method and 99.2–100.2 *per cent.* with Fehling's solution. This recovery must be considered highly satisfactory for the simple fermentation technique used, and it is of special interest that both methods are equally applicable in a quantitative way to measure maltose fermentation, despite the wide discrepancies between the two when reducing substances other than maltose are in question.

Amylases in Malt Extract.—The D.P. and α_0 values of the malts used are shown in Table IV, determinations having been made with ferricyanide under the conditions described in Part I (this *Journ.*, 1949, 103). Mashings were carried out at 10 *per cent.* concentration (50 gm. malt with 500 ml. water) and at 20 *per cent.* (50 gm. with 250 ml.) and at temperatures within the range 61–67° C. (141.8–152.6° F.) for periods of two hours. The appropriate volume of water was taken at such temperature that, when mixed with the malt, the desired temperature was attained in the mash, being thereafter maintained in a thermostatically-controlled water-bath. At intervals, 2-ml.

TABLE II
INFLUENCE OF $\beta : \alpha$ RATIO AND OF TEMPERATURE ON PRODUCTION OF FERMENTABLE
AND UNFERMENTABLE REDUCING GROUPS; 1-HOUR CONVERSIONS
(Results expressed as apparent maltose *per cent.* of starch)

Tem- pera- ture (° C.).	Relative total concen- tration of enzymes.	$\beta : \alpha$ ratio (Cu values).	Before fermentation.		After fermentation.		Fermentable.	
			Fe.*	Cu.†	Fe.	Cu.	Fe.	Cu.
63	X10	0 : 1	45.0	40.0	30.0	23.5	15.0	16.8
		2½ : 1	72.4	69.1	14.3	—	58.1	—
		3¾ : 1	73.8	71.0	12.2	—	61.6	—
		5 : 1	76.1	73.5	11.0	—	65.1	—
		6½ : 1	77.4	75.9	9.6	—	67.8	—
	X20	0 : 1	48.9	44.8	30.2	24.2	18.7	20.6
		2½ : 1	77.9	74.5	10.0	—	67.9	—
		3¾ : 1	78.3	75.0	9.1	—	69.2	—
		5 : 1	79.7	76.0	8.8	—	70.9	—
		6½ : 1	80.6	77.6	8.8	—	71.8	—
65	X 5	0 : 1	37.4	32.6	26.2	20.5	11.2	12.1
		2½ : 1	46.4	42.0	22.4	17.6	24.0	24.4
		3¾ : 1	48.3	43.0	22.0	16.5	26.3	26.5
		5 : 1	53.8	49.5	20.6	16.1	33.2	33.4
		6½ : 1	57.5	52.7	19.1	14.7	38.4	38.0
	X10	0 : 1	43.3	39.0	28.6	22.9	14.7	16.1
		2½ : 1	66.7	62.4	18.3	—	48.4	—
		3¾ : 1	69.0	66.1	14.7	—	54.3	—
		5 : 1	69.9	66.5	13.5	—	56.4	—
		6½ : 1	71.7	67.9	13.3	—	58.4	—
	X20	0 : 1	47.3	42.9	30.0	24.0	17.4	18.9
		2½ : 1	71.3	66.7	17.7	—	53.6	—
		3¾ : 1	74.1	69.8	15.5	—	58.6	—
		5 : 1	75.4	71.7	13.6	—	61.8	—
		6½ : 1	75.9	71.4	12.1	—	63.8	—
67	X10	0 : 1	41.1	36.5	28.6	22.1	12.5	14.4
		2½ : 1	46.7	40.8	27.2	21.2	19.5	19.6
		3¾ : 1	52.6	47.2	24.2	18.5	28.4	28.7
		5 : 1	55.9	51.5	21.9	17.3	34.0	34.2
		6½ : 1	60.4	56.9	20.2	15.2	40.2	41.7
	X20	0 : 1	45.4	40.9	29.0	23.8	16.4	17.1
		2½ : 1	60.9	57.2	21.8	—	39.1	—
		3¾ : 1	64.1	60.0	20.4	—	43.7	—
		5 : 1	68.2	64.5	17.4	—	50.8	—
		6½ : 1	72.8	69.8	13.8	—	59.0	—

* Ferricyanide method.

† Fehling's titration.

portions of the wort were withdrawn and added to 100 ml. soluble starch to bring about conversion under the standard conditions described earlier (21° C., p_H 4.6, 1 *per cent.* soluble starch; *cf.* Part I). Conversion time was 30 minutes, at the end of which aliquots were withdrawn for immediate adjustment to p_H 10.5–11 and determination of reducing groups by ferricyanide titration.

Control was similarly run on each withdrawn mash aliquot. Reducing groups from starch, corrected for those introduced from the mash, give a measure of the diastatic activity of the mash at the time of sampling. The mash was stirred at intervals, these being chosen to allow settling of the grains before withdrawal of aliquots.

The results fall into two groups. Thus, in

Table V are shown the influences of temperature and mash concentration on the survival of amylolytic activity with constant D.P. and α_0 . Table VI shows the influence of

D.P. for two malts when mash concentration and temperature were constant.

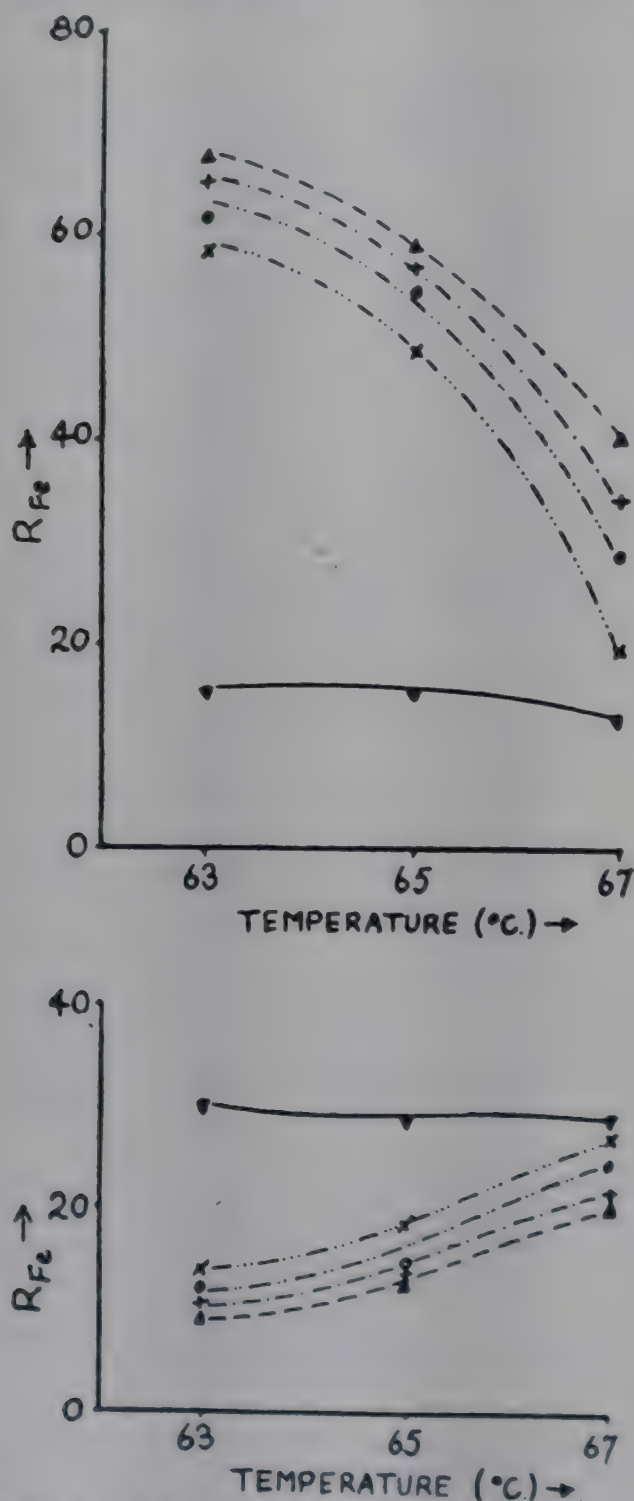
TABLE IV
D.P. AND α_0 VALUES OF MALTS
(Results as ° L.)

Malt.	D.P.	α_0 .	β (by difference).
S3	45.3	9.8	35.5
S4a	101.0	12.3	88.7
S4b	70.8	11.8	59.0
S4c	55.1	11.4	43.7
S4d	42.0	9.8	32.2
S5a	74.1	7.9	66.2
S5b	42.1	7.7	34.4
S5c	29.6	7.5	22.1

DISCUSSION

It will be observed from Tables II and III that total and fermentable reducing groups do not run accurately parallel. Thus, with the X20 total enzyme concentration and with $2\frac{1}{2}$, $3\frac{1}{2}$, 5 and $6\frac{1}{2}$ β units per unit of α , fermentable reducing groups are respectively 75.2, 78.1, 81.9 and 84.1 *per cent.* of total reducing groups in 1-hour conversions at 65° C.; for 2-hour conversions, the corresponding figures are 76.4, 80.7, 84.2 and 86.1 *per cent.* respectively. Hence, the proportion of fermentable reducing groups at constant α concentration increases both with the proportion of β -amylase and with the conversion time; the additional hour of conversion has produced approximately 2 *per cent.* (on total) more fermentable reducing groups, doubtless due to the slow action of the α -amylase.

The influence of temperature is again clear, increasing for constant α as the proportion of β -amylase decreases in the range investigated. Thus, for fermentable reducing groups (X10 concentration, Figs. 1 and 2), the difference between $6\frac{1}{2}$:1 and $2\frac{1}{2}$:1 ratios at 63° C. is 10 *per cent.* (on the starch), whereas at 67° C. the difference is 20 *per cent.* For unfermentables, the influence of either temperature or ratio is less marked, the difference for the highest and lowest ratios being 5 *per cent.* at 63° C. and 7 *per cent.* at 67° C. Thus, to increase the conversion temperature is equivalent to using the original temperature with a lower β : α ratio so far as fermentables are concerned, but the effect on unfermentable reducing groups is less, so small in fact as to have probably no practical



FIGS. 1 and 2—Influence of temperature on production of fermentable (Fig. 1, upper) and unfermentable (Fig. 2, lower) reducing groups. In Fig. 1, reading downwards, the β : α ratios are: $6\frac{1}{2}$:1, 5:1, $3\frac{1}{2}$:1, $2\frac{1}{2}$:1 and 0:1. Total concentration X10. Conversion time 1 hour; p_H 5.4. In Fig. 2, the curves representing the various ratios are in the reverse order.

TABLE III

INFLUENCE OF $\beta : \alpha$ RATIO AND OF TEMPERATURE ON PRODUCTION OF FERMENTABLE AND UNFERMENTABLE REDUCING GROUPS; 2-HOUR CONVERSIONS

(Results expressed as apparent maltose *per cent.* of starch)

Temperature (° C.).	Relative total concentration of enzymes.	$\beta : \alpha$ ratio (Cu values).	Before fermentation.		After fermentation.		Fermentable.	
			Fe.*	Cu.†	Fe.	Cu.	Fe.	Cu.
63	X10	0 : 1	46.6	41.0	29.9	23.8	16.7	17.2
		2½ : 1	72.9	69.4	13.0	—	59.9	—
		3½ : 1	74.2	71.3	11.5	—	62.7	—
		5 : 1	76.5	73.9	10.7	—	65.8	—
		6½ : 1	77.9	76.1	9.3	—	68.6	—
	X20	0 : 1	50.8	46.7	29.8	24.1	21.0	22.6
		2½ : 1	78.8	74.7	9.6	—	69.2	—
		3½ : 1	79.7	76.1	9.2	—	70.5	—
		5 : 1	80.6	76.8	8.5	—	72.1	—
		6½ : 1	80.6	77.3	8.6	—	72.0	—
65	X 5	0 : 1	38.7	34.4	26.8	21.1	11.9	13.3
		2½ : 1	48.7	43.8	23.7	18.4	25.0	25.4
		3½ : 1	51.0	45.8	23.1	18.0	27.9	27.8
		5 : 1	55.6	50.6	20.2	16.0	35.4	34.6
		6½ : 1	61.1	57.7	17.1	13.3	44.0	44.4
	X10	0 : 1	44.9	39.9	29.1	23.4	15.8	16.5
		2½ : 1	68.5	64.2	17.2	—	51.3	—
		3½ : 1	70.4	66.6	15.3	—	55.1	—
		5 : 1	71.7	67.7	13.2	—	58.5	—
		6½ : 1	72.2	68.0	12.9	—	59.3	—
	X20	0 : 1	48.5	44.3	29.8	23.7	18.7	20.6
		2½ : 1	71.7	67.6	16.9	—	54.8	—
		3½ : 1	74.5	69.9	14.4	—	60.1	—
		5 : 1	76.4	73.5	12.1	—	64.3	—
		6½ : 1	76.8	74.3	10.7	—	66.1	—
67	X10	0 : 1	41.4	37.0	28.1	22.3	13.3	14.7
		2½ : 1	47.1	41.5	26.4	20.9	20.7	20.6
		3½ : 1	53.6	48.5	22.3	17.6	31.3	30.9
		5 : 1	56.8	52.2	20.8	16.6	36.0	35.6
		6½ : 1	61.3	57.4	19.4	14.5	41.9	42.9
	X20	0 : 1	46.3	42.3	28.9	23.8	17.4	18.5
		2½ : 1	62.2	58.5	20.9	—	41.3	—
		3½ : 1	64.5	60.6	19.8	—	44.7	—
		5 : 1	70.0	66.5	16.3	—	53.7	—
		6½ : 1	73.7	70.5	13.0	—	60.7	—

* Ferricyanide method.

† Fehling's titration.

significance. This is in complete accord with the view that the fermentable sugars are produced by hydrolysis from the ends of starch chains, this leaving the number of reducing groups in the chains themselves unaltered. However, the numbers of such chains available for subsequent attack diminish as the $\beta : \alpha$ ratio rises; presumably this implies substantially complete disruption of an increasing proportion of attackable chains as the proportion of β -amylase rises.

Wherever possible in Tables II and III both ferricyanide and copper-reducing values have been given; it thus becomes possible in a number of cases to calculate the value of f (Part I, *loc. cit.*). For conversions with α -amylase alone, the range of reductions obtained (copper values) is 32.6 ($f = 1.148$) to 46.7 ($f = 1.085$), the f value throughout the range agreeing very closely with those calculated from the observed reductions by means of the equation $f = 1.292 - 0.00436R_{Cu}$ (Part I, *loc. cit.*); thus, for the reduction extremes of 32.6 and 46.7 *per cent.* the calculated values are 1.150 and 1.088, agreement being excellent. Thus, with the concentrations of α -amylase used in the present investigation the equation, though derived for conversions at 21°C., can be utilized under these conditions of working at high temperature, all aspects of the α -amylase action being apparently similarly affected by temperature increase; with minimal concentrations of α -amylase, however, other considerations supervene (Part II, this *Journ.*, 1949, 298).

The f values for unfermentable material are also of great interest, and the mean of 14 values available for α -amylase conversions is 1.254 ± 0.006 . Agreement is exact with the value previously found in the earliest stages of α -amylase conversion (1.253 ± 0.006). It would appear, therefore, that the special properties of the dextrans with respect to ferricyanide depend little if at all on the actual complexity of the molecule, but rather on the relative proximity of a small number of glucosidic linkages to the terminal reducing group. In the case of joint action, 16 values for the f value of unfermentables give a mean of 1.289 ± 0.007 . This is significantly higher than the value with α -amylase alone, and it is tempting to speculate that in presence of β -amylase a greater proportion of 1-6 linkages is brought into sufficient proximity to end reducing

groups. However, this must await further investigation.

In the case of fermentable materials, the mean f values for α -amylase and joint conversions are, respectively, 0.915 and 0.998. The former low value suggests strongly that appreciable amounts of glucose are formed in the range of conversion investigated; the production of glucose in α -amylase conversions was reported by A. R. Ling and B. F. Davis (this *Journ.*, 1902, 475) who used "restricted diastase" (in effect, α -amylase), and more recently by K. Myrbäck (*Biochem. Z.*, 1940, 307, 140). However, it is not proposed to stress the present indirect evidence—as by attempting an estimate of the relative proportions of glucose and maltose formed, and the question is made more complex by the unknown behaviour of the trisaccharides which are probably present. It may be noted, however, that trisaccharides even if formed might well escape fermentation in the present methods of working (*cf.* Myrbäck, *loc. cit.*; J. Blom and B. Schwarz, this *Journ.*, 1947, 302). What is clear is that (a) the fall in f values during protracted conversions is due to the "diluting" effect of the fermentable materials on the dextrans, (b) it is the preponderating production of maltose which gives a value approaching unity for fermentables when joint conversions are examined, and (c) that the quoted equation relating f and R_{Cu} should not be used for joint conversions.

Further representation of the results of Table II is made in Fig. 3, where fermentable and unfermentable (ferricyanide-) reducing groups are plotted against equivalent D.P. for the various $\beta : \alpha$ ratios. The results are of very great interest. Thus, for ratios of $3\frac{1}{2} : 1$ and greater, production of fermentable reducing groups is substantially independent of ratio. At a given D.P. value below approximately 15, $2\frac{1}{2} : 1$ gives more fermentable reducing groups than do the higher ratios; above this figure it gives inferior results. There is an inflection in the neighbourhood of 55 *per cent.*, but beyond that production increases with rise in D.P. at the rate of approximately 2 *per cent. per 10°* Lintner. Production of unfermentable reducing groups is somewhat differently affected. Thus, results with the various ratios are substantially the same up to D.P. 10–15, but above that the *per cent.* of unfermentable reducing groups remains

almost constant at a level dependent on the β : α ratio. The highest constant level shown, namely for the $2\frac{1}{2}$:1 ratio, corresponds with dextrans themselves having a

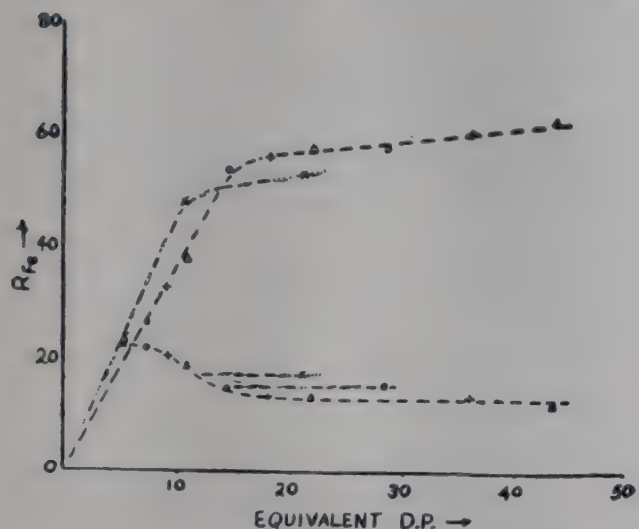


FIG. 3—Influence of β : α ratio on reducing group production at various D.P. equivalence values. For fermentables, ratios of $3\frac{1}{2}$:1 and greater give a single curve (uppermost curve at D.P. 20); at this D.P., the curve for $2\frac{1}{2}$:1 is immediately below. The lowest curve represents unfermentables for 5:1 and greater; above it lie successively at D.P. 20 curves for unfermentables at $3\frac{1}{2}$:1 and $2\frac{1}{2}$:1.

reducing power of the order of 35–36 per cent.; here, for a given D.P., the proportion of α -amylase is greatest, and the dextrans show the least complexity. With the lowest level (5 :1 and $6\frac{1}{2}$:1), the corresponding reducing powers are 30–35 per cent., the proportion of α -amylase being smaller and the dextrans, accordingly, more complex. That unfermentable reducing groups remain constant while fermentables increase, again accords with the view that the latter are split off from the ends of chains, the numbers of chains—and hence of their reducing groups—being unaffected. The copper-reduction reducing powers of the dextrans at this “stable dextrin stage” can be shown to be 24–29, corresponding with an average molecular size of 8–7 glucose units.

Further, if it is assumed that the fermentable reducing material produced in α -amylase conversions is predominantly maltose (an assumption which for the present purpose can involve but little error), it can similarly be shown that the reducing powers (copper values) of the unfermentable dextrans are again 24–30 per cent., so that the average molecular complexity is 8–7 glucose units as

before. It may be recalled that Myrbäck (see, e.g., *Advances in Carbohydrate Chemistry*, 1948, 3, 273) finds the average degree of polymerization of the α -dextrans at the inflection stage to be 7, with which the present figures agree. Since, therefore, in presence of β -amylase (i.e., in joint conversions) the absolute amount of dextrans is decreased through their average molecular size remains unchanged, this can only mean that a proportion of the dextrans is completely hydrolyzed to fermentable material by β -amylase. Such doubtless will be the normal α -dextrans of Myrbäck (see, e.g., *Proc. Eur. Brewery Convention*, 1947, 63); the anomalous (branched) α -dextrans of this complexity, it would appear, are not attacked by β -amylase, a finding which—if confirmed—should provide information as to the points of attack on the amylopectin molecule by α -amylase itself. On the other hand, the attack by β -amylase on the normal dextrans is so rapid as to leave substantially no products of complexity intermediate between these and fermentable sugars. Thus, the starch products formed under mashing conditions would appear to be glucose, maltose and probably trisaccharides (a maltose isomer is not excluded), together with a limit or stable dextrin mixture with average molecular size of 7–8 glucose units, this mixture being slowly attackable by α -amylase with some of its components unattackable by β -amylase. It may be suggested that the true inflection of dextrinization corresponds to a reducing power in the dextrans of approximately 24 per cent. (mean molecular size approximately 8 glucose units), the higher figures usually reported (30–35 per cent. as maltose) being due to the simultaneous occurrence of saccharification to an extent dependent on the actual α -amylase concentration.

Turning now to the survival of amylases in malt extracts, reference may be made to the results of Tables V and VI, selected figures from which are shown by way of illustration in Figs. 4 and 5. Except with the highest activities, where the initial stages are passed through more rapidly than can be followed by this experimental technique, the amylolytic activity of the worts is seen to rise to a maximum, fall off sharply and then, after 40–60 minutes, show a linear decline. It had been supposed that extrapolation of the linear portion back to zero time would give a measure of the initial α -activity of the

TABLE V
INFLUENCE OF MASH CONCENTRATION AND OF TEMPERATURE ON THE SURVIVAL OF
AMYLOLYTIC ACTIVITY
(Malt S3; results are expressed as mgrm. of apparent maltose)

Mash concentration per cent.	Temperature (° C.).	Conversion.	Mashing time (minutes):								
			5	10	15	20	40	60	80	100	120
10	61	A*	64.6	79.6	87.6	90.3	94.9	98.7	101.4	102.3	102.3
		B†	165.0	196.0	215.7	222.3	196.9	174.4	162.8	159.5	150.3
	63	A	52.5	75.6	85.8	89.5	95.9	98.7	99.6	100.5	101.4
		B	112.3	163.1	171.4	162.4	119.5	98.3	88.2	78.1	70.3
	65	A	49.8	73.8	84.9	88.5	93.1	95.9	96.8	97.7	97.7
		B	79.1	104.8	107.8	99.3	67.3	50.9	40.5	30.4	28.1
	67	A	52.6	73.6	80.9	82.6	89.9	90.8	91.7	92.6	93.5
		B	77.0	91.1	79.3	73.0	38.5	26.3	14.0	8.6	3.1
20	61	A	124.1	142.1	161.9	170.9	181.7	188.9	194.3	199.6	205.0
		B	316.3	375.0	382.1	386.5	371.2	339.4	313.7	285.9	262.5
	63	A	118.7	143.9	160.1	170.9	179.9	185.2	193.5	196.1	197.9
		B	290.3	341.6	338.8	319.1	260.5	219.3	179.6	158.9	134.7
	65	A	113.2	147.5	160.1	167.3	178.1	185.3	188.9	192.5	194.3
		B	214.8	292.9	275.9	246.2	163.4	115.7	94.1	68.1	46.0
	67	A	73.8	134.9	156.5	163.7	174.5	181.7	183.5	185.2	187.1
		B	146.3	175.2	162.6	141.8	81.6	51.9	32.1	16.9	1.5

* Reducing groups per 2 ml. of wort.

† Reducing groups liberated from starch, corrected for A, and hence proportional to amylolytic activity.

TABLE VI
INFLUENCE OF D.P. ON THE MASHING SURVIVAL OF AMYLOLYTIC ACTIVITY
(20 per cent. mashes at 65° C.; reducing groups are expressed as mgrm. of apparent maltose)

Malt.	Conversion.	Mashing time (minutes):								
		5	10	15	20	40	60	80	100	120
S4a	A*	128.6	155.4	171.5	180.5	193.8	200.1	203.7	207.3	210.9
	B†	566.7	478.3	395.3	323.8	225.5	183.5	162.1	140.6	119.2
S4b	A	126.1	155.5	171.5	180.5	191.1	196.5	199.2	201.9	204.6
	B	409.4	322.9	285.8	245.5	192.5	155.9	135.2	114.6	93.2
S4c	A	120.7	155.5	169.7	176.9	185.8	192.9	196.5	199.2	201.9
	B	285.6	257.2	231.7	202.2	162.1	128.1	111.2	92.8	74.5
S4d	A	114.4	148.3	167.9	173.3	184.0	194.7	199.2	200.1	201.0
	B	228.9	213.0	191.2	183.5	143.9	115.2	92.8	78.5	64.3
S5a	A	122.4	159.0	167.9	175.1	193.0	196.5	200.1	203.6	207.2
	B	283.6	309.5	277.2	253.2	190.2	156.9	120.9	104.1	87.1
S5b	A	113.4	150.1	162.5	169.1	185.7	191.2	194.7	198.3	201.8
	B	172.0	193.3	180.9	162.2	130.8	107.6	90.6	69.2	47.9
S5c	A	103.6	146.5	158.9	166.1	183.9	188.4	191.2	194.8	198.3
	B	130.1	156.7	148.8	139.3	101.4	83.6	67.4	50.4	38.0

* Reducing groups per 2 ml. of wort.

† Reducing groups liberated from starch, corrected for A, and hence proportional to amylolytic activity.

malt, and that a "humped" portion above this line in the early stages would indicate β -amylase activity. For a single malt at one temperature these ideas appear to be justified; thus (Fig. 4, 61° C.), the apparent "initial" and "final" apparent α -values are approximately twice for a 20 per cent. mash what they are for a 10 per cent. mash. The expected " β -hump" is observed and disappears at 40–60 minutes, suggesting that

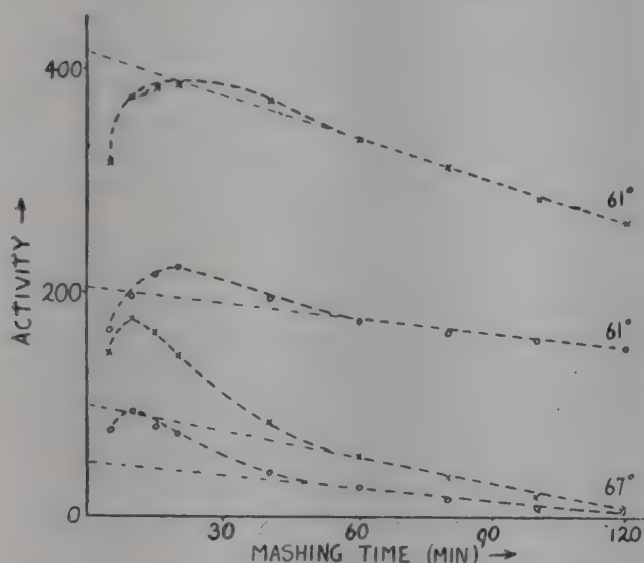


FIG. 4—Survival of malt amylase activity in a single malt mashed at 61° and 67° C., both at 20 per cent. (upper of each temperature pair), and 10 per cent. concentration. Activity is given in arbitrary units.

this enzyme does not survive beyond that time. That it survives so long is surprising; possibly slowness of extraction from the malt affords some protection, though the possibility that protective substances exist in the mash cannot be overlooked. On the other hand, at 67° C. substantially the whole of the amylase— α and β —has disappeared at the end of two hours, and the extrapolated "initial" α -activity is less than one-quarter the value deduced for each concentration at the lower temperature. Thus, so far as α -amylase is concerned, the preliminary explanation is not in accord with the facts.

Fig. 5 is instructive in this connection, showing the results for malts S5a, S5b and S5c; S4d is also included since it has the same D.P. as S5b but a higher α -value. On the other hand, the three malts of series S5, despite their differing D.P. values, have substantially the same α -value (Table IV). In all cases the " β -hump" is observed, terminating as before at 40–60 minutes. The

four malts thereafter show parallel decreases in wort amylolytic activity, the final and "initial" values for the S5 malts coming out in the order of D.P., the activities at a given point being unrelated to the α -activity as determined in the malt. S4d gives activities slightly above those of S5b, presumably related to the higher α -value of the former. Thus, the amylolytic activity in the mash at any given time is profoundly influenced by the β -amylase content of the original malt, and only to a minor extent by its α -amylase content. There is no reason to doubt that β -amylase activity has entirely disappeared after 40–60 minutes; there is equally no reason to doubt that high β -amylase activity in a malt favours α -amylase survival, though the effect is not necessarily direct or entirely due to β -amylase itself.

Thus, there have been many indications that α -amylase stability is reduced by dextrans of intermediate complexity; β -amylase might enhance stability by partial hydrolysis of these (*cf.* Part II, *loc. cit.*). Again, however, malts of high D.P. (which indicates high β -activity) would be expected to contain a high proportion of proteolytic

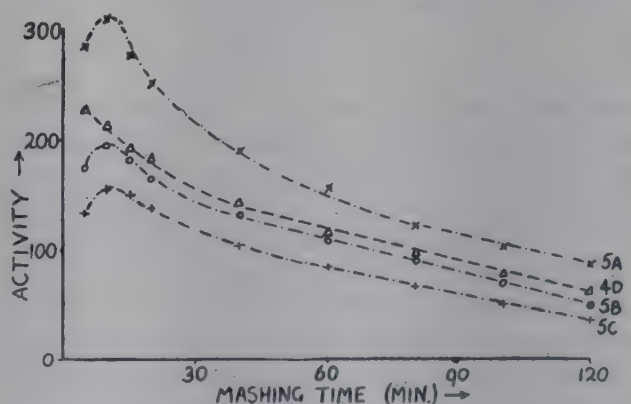


FIG. 5—Influence of D.P., β : α ratio, and α -amylase on amylase survival during mashing (65° C., 20 per cent. mash). The characters of the four malts are given in Table IV.

enzymes which, by proteolysis, would decrease the amount of coagulable nitrogenous matter present and so protect against loss by adsorption (*cf.* Preece, this *Journ.*, 1948, 141). Further, proteolysis might produce protective amino-groups (*loc. cit.*), though appreciable proteolysis to this level is hardly to be expected in an infusion mash. Other possible explanations may suggest themselves, but there seems little doubt that the

distiller's partiality for malts of high D.P. must depend much more on their enhanced α -amylase survival than on any extra liberation of reducing groups during mashing.

The proportion of reducing groups in the samples of wort withdrawn for amylase determinations (figures A in Table V and VI) gives a measure of the degree of starch conversion in the wort. It is surprising that, over the 2-hour mashes studied, there is little difference observed between results with the most active (D.P. 101, 210.9 mgrm.) and least active (D.P. 30, 198.3 mgrm.) malts studied. Assuming an average malt to contain 57 per cent. of starch (*cf.* Preece, *J. Incorp. Brewers Guild*, 1941, 27, 19), these figures correspond roughly to 92.5 and 87.0 per cent. apparent conversion of starch; for true conversion figures, however, they should be decreased by some 5-7 per cent. to allow for reducing sugars preformed in the malt and for invert sugar produced in the mash from sucrose. Both corrections are of uncertain magnitude, but it is certain that some reduction of ferricyanide may take place by non-carbohydrate concomitants. It appears, nevertheless, that rather greater conversion occurs in such mashes than in equivalent starch conversions such as are quoted in Tables II and III. This must be due to the more prolonged action of β -amylase which, it will be observed, allows rapid action to occur up to 40-60 minutes, this supporting the evidence of the activity figures in soluble starch conversion. Thus, although it is felt that considerable reliance can be placed on the relative results obtained for starch conversions using mixtures of the two amylases, the degree of conversion under mashing conditions may, in fact, be some 3-4 per cent. higher than that found by the simple laboratory technique with soluble starch as substrate.

GENERAL CONCLUSIONS

It will be convenient at this point to summarize the principal conclusions reached as a result of this investigation (Preece, this *Journ.*, 1947, 154; 1948, 141; Preece and Shadaksharaswamy, *Biochem. J.*, 1949, 44, 270; this *Journ.*, 1949, 103, 298; also present paper).

1. D.P. is not a reliable guide to the α -amylase content of a malt, but finished malts with high D.P. tend to have a high α -amylase content; the average ratio of

D.P.: α in finished malts is approximately 5:1 (β : α = 4:1), though seasonal, district and varietal variations may well occur in this figure.

2. The final value of the ratio D.P.: α (and therefore of β : α) depends in part on the characters of the barley, in part on general malting treatment, and in major part on the intensity of kiln treatment.

3. At mashing temperature (*e.g.* 149° F.), β -amylase is active for no more than 15 minutes in laboratory starch conversions of the type investigated, but it may persist for 40-60 minutes in malt extracts, this probably resulting in a rather greater degree of hydrolysis than is obtainable in amylase-starch mixtures in the laboratory.

4. α -Amylase is active for prolonged periods in laboratory starch conversions, but activity is lost more rapidly in malt extracts. Persistence of α -amylase activity is favoured by the use of malts of initially high D.P.

5. The amylolytic activity in a mash at a given time is profoundly influenced by the initial D.P. of the malt, and only to a minor extent by the α -amylase content of the malt.

6. Susceptibility to temperature change in mashing is greatest under conditions of low D.P. and low β : α ratio. This is shown particularly by the influence of temperature on the production of fermentable reducing groups. The influence of temperature change within the range investigated on the type of unfermentable dextrans is probably slight from the practical brewing point of view.

7. When the proportion of total amylases to starch exceeds that equivalent to a malt of D.P. 15, the ratio of β : α amylase (so long as it exceeds about 3½:1) is without influence on the production at a given D.P. of fermentable reducing groups, working at mashing temperature. The proportions of fermentable reducing groups formed are then functions of the D.P. of the malt, and a knowledge of this is of more value than of the individual amylase contents (*cf.* conclusion 5 above).

8. On the theoretical side, great care must be exercised in interpreting amylase activity results when ferricyanide is used for determination of reducing groups, as this reagent gives high results for starch conversions brought about in presence of α -amylase.

9. From indirect evidence, the starch hydrolysis products in wort are glucose and maltose together with unfermentable normal and anomalous α -dextrins (maltodextrins); the normal dextrins are particularly susceptible to β -amylase action, but the anomalous dextrins are unattacked by β -amylase and only slowly attacked by α -amylase. The unfermentable dextrins (stable dextrin) produced by α -amylase either in absence or in presence of β -amylase in short period conversions have a mean molecular size equivalent to 7-8 glucose units. The possibility of the presence in wort of trisaccharides and of a maltose isomer is not excluded.

10. Simple laboratory experiments of the type described cannot fully simulate the conditions of brewery mashing, and a complete knowledge of the problem involved cannot be hoped for until there is a full understanding of other concurrent reactions in the mash tun; for it must be understood that the various reactions react upon one

another—possible effects of proteolysis on α -amylase activity have been discussed, and the effects of ionic interchanges between malt and liquor salts (*e.g.*, the behaviour of the calcium ion) are worthy of study. Until there is more information on these and other points—as, *e.g.*, the influence of starch type and concentration—care should be exercised in attempting to apply these results to mash tun conversions. Nevertheless, it is felt that there is here a framework of information which should assist in solving this fundamental problem in brewing chemistry.

Our best thanks are due to Mr. C. M. Lowe, for the careful carrying out of a series of confirmatory experiments on the mashing survival of amylases. We are also grateful to certain Edinburgh breweries for generous supplies of barley and malt.

Heriot-Watt College, Edinburgh.

June, 1949.

UTILIZATION OF AMINO ACIDS DURING YEAST GROWTH IN WORT

By E. C. BARTON-WRIGHT, D.Sc., F.R.I.C., and R. S. W. THORNE, Ph.D.

Pale ale wort has been assayed microbiologically for 16 amino acids both initially and after yeast growth for periods up to 96 hr. Amino acid assimilation represents ~ 70 per cent. of the total nitrogen uptake, arginine making the greatest individual contribution. The amino acid content of yeast remains remarkably constant although grown on different media, and relative uptake from wort of 6 amino acids correlates closely at 24 hr., less closely at 96 hr., with their relative proportions in the yeast, thus supporting the theory of intact assimilation (this *Journ.*, 1949, 201); the theory is further supported by the rapid assimilation of lysine and histidine, amino acids which yeast cannot deaminate.

AN investigation of the uptake of numerous amino acids from wort by yeast, carried out by the technique of microbiological assay, has recently been published by one of us (Barton-Wright, *European Brewery Convention*, 1949); since this work, although not planned with that end in view, has some relevance to the recently proposed hypothesis of intact assimilation of amino acids (Thorne, this *Journ.*, 1949, 201) it seemed of interest to put forward an analysis of it from this standpoint.

Three pale ale worts of specific gravity 1.040 were assayed for 16 amino acids at various time intervals during the course of fermentation with a top yeast. The averaged results are given in Table I, where the amino acids are expressed in terms of their total nitrogen contents in gm. *per* litre. The only important amino acids not represented in this table are glycine, alanine and hydroxyproline, for which microbiological methods are not yet available. The presence of glycine and alanine can, however, be demonstrated

in wort chromatographically and both appear to be largely assimilated by yeast. The total nitrogen assimilation from the 16 amino acids studied is 0.222 gm. *per* litre and it will be shown that this represents about 70 *per cent.* of the total nitrogen assimilation from all sources. The figures in the last column of Table I show that by far the greatest single contribution to yeast nitrogen is made by arginine; lysine is next in importance. Cystine, on the other hand, is

certainly methionine—are present in sub-optimal concentrations.

Now it is an obvious corollary of the hypothesis of intact assimilation that amino acids should be taken up by yeast from wort in approximately the same relative proportions as they occur in yeast proteins. Determinations of some amino acids in yeast grown in several different media have been made (Barton-Wright, *loc. cit.*) and show, as might be expected, a remarkable constancy in their

TABLE I

ASSIMILATION FROM PALE ALE WORT (SP.GR. 1.040) OF NITROGEN IN THE FORM OF VARIOUS AMINO ACIDS DURING TOP FERMENTATION

	Initial nitrogen (gm. <i>per</i> litre).	Nitrogen (gm. <i>per</i> litre) assimilated after:					Nitrogen assimilation <i>per cent.</i> on:	
							Initial individual amino acids.	Total amino acid assimilation.
		9 hr.	24 hr.	48 hr.	72 hr.	96 hr.		
Valine ..	0.0188	0.0013	0.0148	0.0174	0.0175	0.0175	93	7.9
Leucine ..	0.0167	0.0018	0.0109	0.0153	0.0156	0.0156	93	7.0
<i>iso</i> Leucine ..	0.0104	0.0063	0.0087	0.0094	0.0094	0.0095	91	4.3
Serine ..	0.0101	0.0021	0.0074	0.0086	0.0083	0.0083	82	3.7
Threonine ..	0.0122	0.0025	0.0098	0.0109	0.0110	0.0110	90	5.0
Aspartic acid ..	0.0076	0.0009	0.0066	0.0070	0.0070	0.0070	92	3.2
Glutamic acid ..	0.0139	0.0023	0.0093	0.0113	0.0115	0.0115	83	5.2
Methionine ..	0.0029	0.0009	0.0029	0.0029	0.0029	0.0029	100	1.3
Cystine ..	0.0016	0.0000	0.0005	0.0010	0.0010	0.0010	63	0.5
Lysine ..	0.0259	0.0162	0.0226	0.0218	0.0219	0.0219	85	9.9
Arginine ..	0.0825	0.0120	0.0631	0.0778	0.0778	0.0778	94	35.0
Proline ..	0.0595	0.0000	0.0041	0.0085	0.0085	0.0085	14	3.8
Phenylalanine ..	0.0066	0.0002	0.0047	0.0062	0.0062	0.0062	94	2.8
Tyrosine ..	0.0067	0.0004	0.0028	0.0061	0.0061	0.0062	93	2.8
Histidine ..	0.0128	0.0015	0.0085	0.0104	0.0107	0.0108	85	4.9
Tryptophane ..	0.0064	0.0004	0.0037	0.0062	0.0063	0.0063	99	2.8
Standard deviation		± 0.0019						
Totals ..	0.2946	0.0488	0.1804	0.2208	0.2217	0.2220	—	—

assimilated to a smaller extent than any other amino acid. The rapid assimilation of lysine and histidine from wort, despite the fact that yeast is unable to deaminate them, has already been adduced (Thorne, *loc. cit.*) in favour of the hypothesis of intact assimilation of amino acids. The figures in the penultimate column of the table show that the only amino acid which is quite obviously present in great excess of its requirements is proline. Most of the amino acids in wort are taken up by yeast to the extent of about 90 *per cent.* of their initial amount. Probably some of them—and

relative proportions regardless of the nutrient medium used. The averaged values of these determinations are given in the second column of Table II, expressed as gm. total nitrogen contributed by the specified amino acid *per* 100 gm. of dry yeast. (Determinations of methionine were excluded for two reasons: (i) the amount of methionine in wort is so small that a good deal of the yeast methionine must necessarily be synthesized *via* ammonia, and (ii) the methionine content of yeast, unlike that of the other amino acids examined, seems to be variable.) The third and fourth columns of Table II show the corresponding

figures for amino acid assimilations from wort after 24 and 96 hr. (taken from Table I). The degree of association between the amino acids removed from wort and the amino acids appearing in the yeast may best be judged by calculating the correlation coefficients, r , between the corresponding sets of figures: these (0.95 after 24 hr. growth and 0.88 after 96 hr.) are shown at the foot of the table. The significances of these values

TABLE II

RELATION BETWEEN SOME AMINO ACIDS IN YEAST AND THEIR ASSIMILATION FROM WORT

	Grm. nitrogen per 100 grm. dry yeast.	Grm. nitrogen assimilated per litre after:	
		24 hr.	96 hr.
Valine ..	0.370	0.0148	0.0175
Leucine ..	0.339	0.0109	0.0156
isoLeucine ..	0.281	0.0087	0.0095
Lysine ..	0.720	0.0226	0.0219
Phenylalanine	0.156	0.0047	0.0062
Histidine ..	0.377	0.0085	0.0108
Correlation between amino acids in yeast and amino acids assimilated.		$r = +0.95$ $P = 1/300$	$r = +0.88$ $P = 1/50$

are expressed by the probability, P , that such values could be obtained by chance from uncorrelated data. It is seen that after 24 hr. growth the association between amino acids assimilated and amino acids in yeast is so close that there is only 1 chance in 300 that the agreement could be fortuitous; after 96 hr. the chance has risen to 1 in 50 but the association remains, even so, highly significant. The close proportionality between amino acid uptake and amino acid content of yeast required by the hypothesis of intact assimilation is thus fully confirmed experimentally. Moreover, in view of the facts that (i) amino nitrogen is being excreted during the later stage of yeast growth, and (ii) some deamination of amino acids is also occurring to supply ammonia for the synthesis of amino acids exhausted in the earlier stages of growth, and perhaps too for the synthesis of some non-protein nitrogenous constituents of yeast [arginine and histidine may, however, be precursors of purines as indicated by Hopkins (*J. chem. Soc.*, 1916, 109, 629)], it may reasonably be expected that a higher correlation between amino acids in yeast and

their assimilations from wort should be found when the assimilations are measured after 24 rather than 96 hr. This again is just what is shown by the experimental results. (The 9-hr. assimilations have not been used since they are rather small and therefore relatively less reliable.)

The assimilations of different classes of nitrogen compounds from two pale ale worts (of mean sp.gr. 1.036) during top-fermentation have also been determined, the averaged results being shown in Table III. These are similar to the previously published results (Barton-Wright, *loc. cit.*) except that the ninhydrin method was used for the determination of α -amino nitrogen. The following observations should be made about the various categories:—

Total Crystalloid Nitrogen.—This was estimated by determining total nitrogen on worts which had been cleared of protein with colloidal ferric hydroxide.

Nitrogen as Amides.—Amide nitrogen was estimated by determining ammonia on the cleared and hydrolysed wort and deducting the initial ammonia nitrogen from the figure so obtained. It is assumed that the amides of wort are of the asparagine and glutamine type and therefore the nitrogen as amides is taken to be twice the amide nitrogen.

α -Amino Nitrogen.—This was determined by the ninhydrin method of Van Slyke, MacFadyen and Hamilton (*J. biol. Chem.*, 1941, 141, 671) which is specific for the α -amino group of free amino acids. The α -amino groups of peptides do not react. Aspartic acid, on the other hand, gives twice the theoretical result. The α -amino nitrogen due to amino acids is obtained by subtracting from the α -amino nitrogen found that due to the α -amino nitrogen of amides.

Nitrogen as Amino Acids.—Some amino acids contain nitrogen other than that in the α -amino group. Now the microbiological assays of Table I can be recalculated as α -amino nitrogen instead of total nitrogen, allowance also being made for the anomalous reactivity of aspartic acid with ninhydrin; it is then found that 0.2220 grm. total amino acid nitrogen in these worts is equivalent to 0.1495 grm. α -amino nitrogen determined by the ninhydrin reaction. Assuming that the same distribution of amino acids holds for the worts in Table III as for those in

TABLE III
ASSIMILATION OF VARIOUS CLASSES OF NITROGEN COMPOUNDS FROM PALE ALE WORT
(SP.GR. 1.036) DURING TOP FERMENTATION

	Initial nitrogen (<i>gram. per</i> <i>litre</i>).	Nitrogen (<i>gram. per</i> litre) assimilated after:					Nitrogen assimilation <i>per cent. on</i> :	
		9 hr.	24 hr.	48 hr.	72 hr.	96 hr.	Initial amount of class.	Total assimi- lation.
Total crystal- loid N ..	0.5350	0.0900	0.2300	0.2550	0.2500	0.2500	47	100
N as ammonia	0.0215	0.0035	0.0175	0.0175	0.0185	0.0185	86	7
N as amides ..	0.0710	0.0120	0.0230	0.0280	0.0290	0.0300	42	12
α -Amino N of amino acids	0.1620	0.0350	0.0970	0.1185	0.1180	0.1175	—	—
N as amino acids ..	0.2406	0.0519	0.1441	0.1761	0.1752	0.1746	73	70
Residual N ..	0.2019	0.0226	0.0454	0.0434	0.0273	0.0269	13	11
Standard deviation		± 0.0008						

Table I, the α -amino nitrogen of amino acids can be converted to total nitrogen of amino acids by simple proportion. This proportionality will be slightly upset by the absence of glycine and alanine from the microbiological assays but the error is not likely to increase the apparent total nitrogen of amino acids by more than 1 or 2 *per cent.*

Residual Nitrogen.—This is the figure obtained by deducting from the total crystalloid nitrogen the nitrogen due to ammonia, amides and amino acids. In the unfermented wort its amount is 38 *per cent.* of the total crystalloid nitrogen and it consists, presumably, mainly of peptides with some purines and pyrimidines.

Turning now to the figures actually recorded in Table III, and especially to the last column which shows the percentage contribution made by each category to the total assimilation, it is seen that no less than 70 *per cent.* of the total assimilation is of amino acids. No doubt the greater part of this is assimilated intact (Thorne, *loc. cit.*).

Ammonia nitrogen accounts for 7 *per cent.* of the assimilation and the nitrogen of amides for another 12 *per cent.*, leaving only 11 *per cent.* unaccounted for. Probably all of this last category should be regarded as peptide assimilation; whether the peptides are assimilated intact or, as is more likely, after hydrolysis to amino acids cannot at present be determined. In any case the net amount of peptide assimilation is a rather small proportion of the total. It is worth noting, however, that it is only in this category of residual nitrogen that strongly marked excretion is observable: after 24 hr. 20 *per cent.* (0.0454 *gram.* residual nitrogen out of 0.230 *gram.* total crystalloid) has been assimilated; that this figure drops to 11 *per cent.* after 96 hr. must be attributed to excretion.

The Brewery, Chiswell Street, London, E.C.1.;
Institute of Brewing Research Laboratories,
Birmingham University.

29th August, 1949.

ABSTRACTS

I.—MATERIALS AND ANALYSIS

Farm Grain Storage. W. A. HAYLES (*Agriculture*, 1949, 56, 228-233). It is estimated that 11,000 combine harvesters will be at work in 1950, and although merchants are installing bulk-storage to hold some of the grain, it will become necessary for farmers to instal equipment capable of storing at least a part of their combined cereals. The grain, if free from green stuff and having a moisture content of 15-16 *per cent.*, may be stored under farm conditions for up to six months. Storage in sacks is efficient if the grain is not damp, but an upper storey is preferable; if a ground floor must be used a temporary slatted floor is advisable. Sack storage is expensive owing to the high cost of sacks and high handling costs. Pre-cast concrete bins are available in 10 sizes up to 15 ft. in diameter and may be erected to any height up to 29 ft. The bins are cheap and readily available, and are easily erected, dismantled and re-erected. A square type of bin is available constructed from hollow pre-cast sections in such a manner that several bins, each 10 ft. square, having common sides, may be installed in a building in the minimum space. A self-emptying hopper-bottom may be fitted if desired. Bins with 9-in. walls may also be constructed from bricks, partitions and corners being bonded. Internal tie-rods need not be used unless the bin is more than 10 ft. high and holds more than 25 tons. Using Flemish bond, the walls may be reinforced by $\frac{3}{8}$ -in. steel tie-rods placed vertically and set in the concrete floor. A damp course should be inserted near floor level. Wooden bins are excellent; they are constructed from 2 x 2 in. or 2 x 3 in. timber. They are economical in space and easily constructed, but are not fireproof and are expensive. Timber bins may also be constructed with a frame of H-section vertical steel stanchions.

Ventilation of the bins may enter through openings or *via* louvres built in the lower parts of the walls. Another method is to construct the bin of specially shaped concrete beams or, alternatively, build air ducts into the bin, but the effectiveness of this method is doubtful. Forced ventilation may be used to prevent overheating or to reduce the moisture content of the grain; it is, however, an unsatisfactory substitute for the grain drier when high moisture contents are encountered. The temperature of the air may be raised slightly if necessary. The bin floor may be of the self-emptying type, but this reduces the capacity by up to one-third and is not necessary on

farms where bins are only occasionally discharged. A conveyor system, mechanical or pneumatic, must be installed and this is a specialist job. In most cases a bulk installation will consist of a building, receiving hopper, grain dresser, bins, conveyors, power units, sacking-off facilities and sack storage space. A. E. W.

Insecticides and Poisoning of Crops.

J. H. BURN (*Chem. & Ind.*, 1949, 601-604). An important question involved in the application of insecticides is their effect on those who handle them and in the earlier periods of use much difference of opinion may be expressed by different investigators. The author quotes examples of the varied conclusions reached by those controlling the use of DDT and similar compounds and discusses the physiological action of TEPP (tetra-ethyl pyrophosphate) in the human body and an account is given of the effect of these compounds, so far as it is known, when introduced into animals compared with historic epidemics of accidental poisoning due to other materials. In the U.S.A., the general public was alarmed by the statements of one observer that the widespread use of DDT was responsible for an epidemic disease, and an official warning was issued by a public department against the supposed dangers of its use whilst a maximum limit was given for the amount of residues of this and other insecticides which should be allowed in foods. Later, a further statement was issued by another government department contradicting the former one and indicating that whilst thousands of tons of DDT have been used in many directions no evidence has ever been obtained that human sickness is due to the use of this insecticide, any minor toxic symptoms observed being probably caused by the kerosene solvents. Little is yet known about the action of insecticides upon the fruit and vegetables to which they may be applied and such knowledge as we possess is very speculative. More attention should be given to the variation in sensitiveness to these compounds shown by different individuals of the same species, since although a large number of individuals may not be affected by a certain concentration of a particular compound a minority may be highly susceptible. It is difficult to assess the comparative efficiency of insecticides, since under apparently similar conditions different results may be obtained owing to variation of factors of which we have no knowledge. Even the same species of insect will exhibit greater or less immunity at different times and different

batches of the same preparation may show varied degrees of toxicity. T. J. W.

Changes in Carbohydrates During Caramelization of Malts. G. CHABOT and M. COSAERT (*Fermentatio*, 1949, No. 2, 33-48). This work was done on a Belgian Chevalier barley and a number of malts of increasing degree of caramelization made from that barley. The extracts on which the sugars were determined were prepared by macerating 10 grm. of the ground malt (or barley) with 40 c.c. of 0.1 N sodium hydroxide for 30 min. at room temperature, neutralizing with 0.1 N sulphuric acid, adding 20 c.c. of Carrez A solution followed by 20 c.c. of Carrez B solution (for clarification), making up to 200 c.c., filtering and re-making the filtrate to 200 c.c. Determinations were made of total reducing sugars, maltose, glucose, fructose, sucrose, dextrins and starch. It was found that the total reducing sugars increased with increasing caramelization from 3.6 *per cent.* in the pale malt to 21 *per cent.* The method of caramelization had an effect, and only under normal conditions of caramelization was there a direct relation between total reducing sugars and colour. This relation held only as far as a colour of 16° L.; caramelization beyond this point (to a colour of 72° L.) produced no further increase in reducing sugars. With maltose there was a sharp rise from 0.517 *per cent.* in the pale malt to 12.58 *per cent.* with the onset of caramelization. There was no regular increase in maltose content as caramelization increased. With glucose there was an increase from 1.78 *per cent.* in the pale malt to 5.51 *per cent.* in malt with a colour of 32° L., followed by a slow, steady diminution as caramelization increased. With fructose there was an increase throughout the whole of caramelization, starting from zero in the pale malt and rising to 2.45 *per cent.* Sucrose was at its maximum in the pale malt (5.1 *per cent.*) and decreased as caramelization increased. The extent of the decrease was influenced by the quality of the caramelized malt; for malts with a colour of 16° L., the best contained 3.96 *per cent.* while the poorest contained only 1.14 *per cent.* of sucrose. Dextrins increased sharply from zero in the pale malt to a value dependent on the method of determination used, then diminished as caramelization became intense. As with sucrose, the higher the quality of the caramelized malt the greater its dextrin content. The changes in starch content showed that this substance is the principal source of the colouring and flavouring substances produced. The starch content diminished rapidly in the early stages, but showed only small variation later.

There follows an account of a brief investigation into the amount of reductones present

in the malts and in certain beers. The reagent used was prepared by adding ammonia to 0.1 N silver nitrate solution until the precipitate first produced was just redissolved. Five drops of this reagent were added to 2 c.c. of a 12 *per cent.* aqueous extract of the malt, and the extent to which darkening or formation of a black precipitate had occurred was noted immediately and after 5, 10 and 15 min.; vitamin C solution was used as a control. The results show that the amounts of reductones in the malts increase with increasing colour, that pale beers contain practically no reductones whilst dark beers contain amounts that are readily detectable, and that substances of a reductone nature may be added to beer at racking or bottling with advantage. A. A. D. C.

Malt Quality as Seen by Maltsters and Brewers. G. KAURT (*Fermentatio*, 1949, No. 2, 17-26). The qualities sought in malts are of two kinds; general qualities, common to all malts, and special qualities, determined by the nature of the malt and the particular type of beer for which it is to be used. The first of the general qualities is dryness, not only because moisture represents loss of extract but because excessive moisture allows undesirable changes to take place in the malt. The second is complete and regular germination. There should not be more than 3-4 *per cent.* of dead corns, and evenness of growth is more important than extent of growth. All malts should, of course, be clean and free from foreign seeds, stones and any infestation. Whether a malt gives a bright or opalescent wort is of minor importance, since this is to a large extent a varietal characteristic and does not necessarily indicate that the malt is undermodified. The special qualities of a malt are degree of modification, colour, saccharification time, aroma, and type of "break" of the wort. The author does not favour chemical methods of assessing modification, which he considers should be judged by physical means. He illustrates the choice of special qualities by describing malts destined for Pilsen beer, Munich beer, top-fermentation beer, and beer brewed with a high proportion of adjuncts. In Pilsen beers malt flavour is subservient to hop flavour, and the malt must therefore have a low nitrogen content and degree of modification and be kilned at a low temperature, in order to keep aroma, sugars and soluble nitrogen to a minimum. In Munich type beers, however, malt flavour predominates, and a malt of higher nitrogen content can be chosen, modified to a higher degree, and cured at a higher temperature, with the object of producing more sugars and soluble nitrogen and a distinctive aroma. The colour in a well made Munich malt is due more to the melanoidins formed on the kiln from the sugars and soluble

nitrogen than to the use of high temperatures. Top-fermentation beers owe their character mainly to the esters formed from the higher alcohols which are produced by de-amination of amino acids, and the worts should therefore be rich in amino acids. To this end the malt must be the result of a slow, lengthy germination, well modified and well cured on the kiln. For beers brewed with a high proportion of adjuncts, the one essential malt quality is a high diastatic power, and malt of a higher nitrogen content than would normally be accepted can be used.

A. A. D. C.

Brewing Value of Malt. B. D. HARTONG (*Amer. Brewer*, June, 1949, 19-20, 58). The author has devised a new method, based on theoretical considerations which were confirmed by experimental work, for determining the brewing values of malts, the results being expressed as evaluation numbers (*E.N.*). Samples of the finely-ground malt are mashed by the official Congress Method at temperatures of 25°, 45°, 65° and 85° C. and the extracts obtained are calculated to percentages of the highest value obtained, the proportional yield thus determined being the solubility of the malt at the particular temperature used for mashing. Preliminary experiments indicated that the differences between the two lower temperature results were dependent upon the amount of enzymes formed whilst those shown between the values obtained at the higher temperatures were due to variation in the attackability of the malt. The results obtained are applied in the following formula:—

$$E.N. = \left(\frac{P_{25^\circ} + P_{45^\circ} + P_{65^\circ} + P_{85^\circ}}{4} \right) - 58$$

in which *P* is the proportional yield at the temperature indicated. The constant 58 was adopted in order to give a series of *E.N.* values ranging from 0 to 10 with 5 as the ideal in the centre of the scale. Normal malts yield results between 2.6 and 6.2, lower values indicating derivation from barley of low germinating power, whilst values above 6.2 are shown by malts with high enzymic activity. The *E.N.* alone is little more than a rough indication of general malt quality but much more information is given by the differences between the results of the two lower temperature mashes and between those of the two higher ones. According to the author's hypothesis every malt has two independent properties, and thus a simple "modification" based upon one only has no real existence. The evaluation numbers are completely additive and the values for any mixture of two or more malts may be calculated accurately from those of the individual constituents. Various chemists who applied this method stated that the analyses

occupied too much time and the author modified it by using mashing temperatures of 40° and 85° C. only and calculating according to the following formula:—

$$E.N. = \left(\frac{2P_{40^\circ} + P_{85^\circ}}{4} \right) - 34$$

which yields the same range of *E.N.* values from 0 to 10. The theoretical aspects of this procedure are dealt with mathematically, and a large number of determinations have shown that the method is applicable to malts from many parts of the world.

T. J. W.

Malt Extraction in Determination of Amylase Activities. V. L. ERLICH and G. M. BURKERT (*Cereal Chem.*, 1949, 26, 326-333). Conventional methods for the determination of diastatic power by extraction with distilled water are sufficiently accurate for comparative purposes when dealing with materials of the same general type, but fail to extract the total quantity of α -amylase from other products, such as wheat-malt, etc. The authors have shown that extraction with dilute solutions of various salts yields an extract exhibiting a higher and more consistent α -amylase activity, and this modification does not necessitate strict adherence to the conditions under which extraction is carried out. Two procedures have been elaborated, of which one, the regular method, yields results which conform with those obtained under technical mashing conditions. *Regular method.*—500 ml. of 0.4 per cent. sodium bicarbonate solution are added to 25 grm. of ground malt, maintained at 30° C. for 2 hr., and then filtered at the ordinary temperature. 10 ml. of the filtrate are diluted to 100 ml., and 10 ml. of the dilute solution are used for determination of the dextrinizing time at 20° C. by the Olson, Evans and Dixon method. Alternatively, the end-point may be determined by reference to the glass colour-standard of Redfern. *Rapid method.*—5 grm. of the ground malt are placed in the bowl of a Waring Blendor containing 500 ml. of 0.4 per cent. solution of sodium bicarbonate at about 35° C. and the mixer is run at a low speed for 10 min. The whole of the infusion is poured into a filter and a suitable aliquot is used for determination of the dextrinizing time. This latter method is not suitable for preparing extracts required for the determination of diastatic power, since the vigorous stirring adopted reduces the β -amylase activity. For wheat malt, neither the diastatic power nor α -amylase determination is a reliable indication of the enzymic activity when used in baking or in the distillation industry, and further investigation is required to discover a suitable extraction procedure.

T. J. W.

Replacement of Rochelle Salt by Glycerol in the Determination of Sugar. S. N. LUTOKHIN and O. A. KUZMENKO (*J. anal. Chem. Russ.*, 1948, **3**, 196-197; through *Analyst*, 1949, **74**, 327). Previous workers who have used glycerol in place of Rochelle salt in Fehling's solution have found that the normal tables could not be used in the higher range of concentrations of sugar. If the procedure given below is followed, the iodimetric method of Fresenius (*Anleit. chem. Anal. Weines*, 1922) gives correct results with concentrations not exceeding 5 grm. per litre and by using Fresenius's tables. *Procedure*.—To 10 c.c. of the sugar solution add 3 c.c. of glycerol, 9 c.c. of 20 per cent. potassium hydroxide solution, 10 c.c. of 4 per cent. copper sulphate solution, and 10 c.c. of water. Then proceed as described in Fresenius's method. A. A. D. C.

Colorimetric Method for Carbohydrates: Maltose. G. H. BENHAM and V. E. PETZING (*Analyt. Chem.*, 1949, **21**, 991-993). The authors have adapted the molybdenum-blue method of Benham and Despaul (this *Journ.*, 1949, **49**) to the determination of mixtures of glucose and maltose. A sample containing not more than 500 mgrm. of the mixed sugars is weighed, dissolved in water and diluted to 100 ml. 50 ml. of this solution are diluted to 100 ml. and 2 ml. of the dilution are transferred to a 25-ml. volumetric flask together with 5 ml. of 0.02 M potassium dihydrogen phosphate and 10 ml. of 7.5 per cent. ammonium molybdate solution, the whole being diluted to the mark and mixed. The flask is placed in a pre-heated autoclave and maintained at 100° C. for exactly 30 min., then removed and placed in ice until cooled to room temperature. The blue liquid is transferred to the cell of a spectrophotometer and the transmittency is determined at a wavelength of 650 m μ . The value of the reading is equivalent to the glucose-maltose mixture present. The remaining 50 ml. of the original solution are added to 3 ml. of 10 per cent. hydrochloric acid and the mixture is heated under a pressure of 15 lb. (121° C.) for 1 hr. to hydrolyse the maltose, and after cooling the liquid is neutralized with 0.1 N sodium hydroxide and diluted to 100 ml. 2 ml. of this solution are submitted to the same procedure as the unhydrolysed solution above and from the two readings obtained the percentages of glucose and maltose present are calculated by means of two standard curves and algebraical equations, of which full details are given in the original paper. When this method is used for the analysis of partially hydrolysed starch products and similar preparations it is essential that any dextrans and oligosaccharides should be removed quantitatively beforehand. The results obtained by this procedure are in close

agreement with those given by the Munson and Walker method for total reducing sugars. T. J. W.

Determination of Starch and Cellulose. F. J. VILES, jun., and L. SILVERMAN (*Analyt. Chem.*, 1949, **21**, 950-953). The authors have investigated the effects of the various factors involved in the following method, which is based on the formation of a green colour by the addition of an acid solution of anthrone to a solution of the carbohydrate, the intensity of the colour produced being proportional to the amount of starch or cellulose present. The reagent used is a 0.10 per cent. solution of anthrone in concentrated sulphuric acid, but this is somewhat unstable and different lots do not exhibit the same degree of sensitiveness, so that no single curve relating the colour depth and the amount of carbohydrate can be used. For this reason one or more known standards, together with a reference blank made with the reagents only, should be included in each set of determinations carried out. Starch determination: the solid is boiled in distilled water and after cooling is diluted so that a 2-ml. aliquot contains from 10 to 200 microgm. of starch. If turbid, the solution is filtered through filter paper, and 2 ml. of the filtrate is transferred to the tube of a colorimeter provided with a logarithmic scale and an orange filter. 4 ml. of the reagent are added rapidly and the mixture is stirred immediately, allowed to stand for 10 or 15 min., and then cooled in water. The colorimeter is adjusted to zero reading with the reference blank and the readings of the samples and standard solutions are then read. Cellulose determination: the solid is digested in 60 per cent. sulphuric acid at the ordinary temperature for 15 to 30 min. and diluted with acid of the same strength so that a 0.5 ml. aliquot contains 10 to 200 microgm. of cellulose. If necessary, the liquid should be filtered through an asbestos mat. A volume of 0.5 ml. is added to 2 ml. of water and after 4 ml. of the reagent has been run in the subsequent procedure is the same as for starch (above). The method may be applied to mixtures of starch and cellulose by making a boiling water extract, filtering, and digesting the residue with 60 per cent. sulphuric acid.

T. J. W.

Colour Reagents for the Paper Chromatography of Sugars. W. G. C. FORSYTH (*Nature*, 1948, **161**, 239-240; through *Analyst*, 1949, **74**, 327). The tests used in the application of paper chromatography to the identification of sugars (Partridge, *ibid.*, 1946, **158**, 270; also this *Journ.*, 1949, **247**) may be made more selective by using resorcinol and naphthoresorcinol as test reagents in addition to the usual ammoniacal silver nitrate reagent. The

procedure is as follows: Three spots of a 1 per cent. solution of the sugar are placed on a filter paper and, alternately, 3 spots of a standard mixture of sugars, and the paper is dried in the usual manner. The paper is cut into 3 strips and the strips are sprayed, one with ammoniacal silver nitrate solution, one with resorcinol solution, and one with naphthoresorcinol solution. The phenolic solutions are prepared by adding 10 c.c. of a 1 per cent. solution of the phenol to 90 c.c. of 2 N hydrochloric acid. The papers sprayed with the phenol solutions are dried at 85° and 95° C. (185° and 203° F.) for 10 min. Colours given by sugars with the phenols are (1) *with resorcinol*: fructose, red; xylose and arabinose, blue; rhamnose, yellow; (2) *with naphthoresorcinol*: galactose, glucose and mannose, grey; fructose, brown; xylose and arabinose, blue; rhamnose, green. Non-reducing sugars also give a positive reaction.

A. A. D. C.

Separation of Organic Acids and Acidic Amino-acids by the Use of Anion-Exchange Resins.

S. M. PARTRIDGE and R. C. BRIMLEY (*Biochem. J.*, 1949, **44**, 513-521). Three commercial anion-exchange resins, Amberlite I.R.4, Wofatit M and De-Acidite B, have been investigated for their suitability for displacement chromatography on a preparative scale. Aspartic acid and glutamic acid were well adsorbed by the resins, and the main object of the work was to establish conditions for the separation of these acids from each other and from mixtures containing neutral amino-acids. The procedures followed were similar to those used by Partridge and Westall when using kation-exchange resins (this *Journ.*, 1949, 324). Titration curves for the three resins, carried out in the presence of neutral salts, show that they have weakly acidic properties in addition to their main function as insoluble bases. For this reason their regeneration must be carefully controlled, and strong alkalis should be avoided when dealing with large columns. De-Acidite B may be regenerated with sodium acetate. The boundary widths given by glutamic acid are approximately inversely proportional to the adsorptive capacity of the resins, and in general the boundaries given by these resins are much broader at useful rates of flow than those given by the Kation-exchanger Zeo-Karb 215. The separation of weak acids can therefore be successful only at very slow rates of flow and with finely-ground resins, and the technique is of doubtful practical value (with the anion-exchange resins at present available), although some separation between glutamic and aspartic acids was achieved on De-Acidite B. The resins are, however, useful for the separation of glutamic or aspartic acid from neutral amino acids, particularly serine. Since the resins

adsorb the neutral amino acids to a small degree only, separation can be achieved by a simple process of adsorptive filtration. Using De-Acidite B, glutamic acid and aspartic acid may be separated on a preparative scale by developing the column with acetic acid. This forms a band lying between and overlapping the bands due to the amino acids.

A. A. D. C.

Fractionation of a Protein Hydrolysate.

S. M. PARTRIDGE (*Biochem. J.*, 1949, **44**, 521-527). A procedure is described for the isolation of amino acids from protein hydrolysates on a preparative scale, using displacement chromatography on ion-exchange resins. Since tyrosine and phenylalanine interfere with the separation, the first step was to remove them (together with soluble humin) by adsorption on charcoal, from which they were recovered by elution with aqueous phenol-acetic acid. In the example given, the charcoal-treated hydrolysate of commercial egg albumin was then fractionated on a column of Zeo-Karb 215 by displacement with dilute ammonia solution. By one passage through the column the mixture of amino acids was separated into seven bands, as shown by analysis of the flowing chromatogram by filter-paper chromatography, the bands containing (1) aspartic acid, (2) glutamic acid, serine and threonine, (3) glycine and alanine, (4) valine and proline, (5) leucine, *isoleucine*, methionine and cystine, (6) histidine and an unidentified amino acid, and (7) lysine. The amino acid or acids contained in each band were recovered as salt-free crystalline solids by evaporating appropriate fractions from the effluent solution, and represented a yield of 45.6 per cent. on protein dry weight. The means to be adopted for the isolation of the pure amino acids from the mixtures in the bands is discussed, and as examples of the procedures suggested three such separations are described. Glutamic acid was isolated from the second band by adsorption on De-Acidite B, a mixture of the isomeric leucines was isolated from the fifth band by selective oxidation with bromine water, followed by further fractionation on ion-exchange columns, and the separation of glutamic acid (and, to some extent, serine) from the second band was also accomplished by displacement from a column of Zeo-Karb 215 by means of a dilute solution of ammonia in 50 per cent. aqueous acetone.

A. A. D. C.

Semi-micro Determination of Uronic Acids. G. G. MAHER (*Analyt. Chem.*, 1949, **21**, 1142-1143). A simply constructed apparatus is described and illustrated. The method of operation depends upon the liberation of carbon dioxide when uronic acids are heated at 160° C. with 12.5 per cent. hydrochloric acid. The carbon dioxide is swept by a stream of pure air through an absorption tower containing

standard barium hydroxide which is then titrated with standard hydrochloric acid. For samples weighing from 11 to 40 mgrm., results are obtainable in from 120 to 450 min. according to the nature of the material under test.

A. E. W.

Methods used for Determination of Water in the Laboratory. A. S. JOHNSON (*Chem. & Ind.*, 1949, 511-514). The author reviews some of the methods used for the determination of moisture and discusses the value of each for certain materials. The principal factors for consideration are the selection of a method suitable for the sample under test, one capable of yielding results in the time available, and the desired accuracy of the results. The method most widely used in the past is gradually being superseded by apparatus consisting of an oven in combination with a balance which enables the dish and sample to be weighed without removal, as in the Brabender tester. Such methods are, however, unsuitable for substances which are affected by heat or contain volatile matters other than water. For samples of this class, the Dean and Starke procedure is suitable, this involving distillation with a liquid with which water is immiscible. The water vapour from the sample is condensed and its volume measured. The use of a vacuum desiccator whether at ordinary or higher temperatures is slow, and may require 24 hr. or more. In this connection it should be remembered that magnesium perchlorate is a more efficient drying agent than calcium chloride which is often used. The Karl Fischer method is a chemical one and depends upon the reaction of the water in the sample with a mixed reagent containing free iodine, the moisture content being deduced from the result of a volumetric titration. For light coloured samples the end-point is determined visually but those of dark colour necessitate an electrometric determination. The procedure is applicable to some substances which cannot be tested in any other way, and although somewhat complicated and requiring careful attention to detail it is capable of high precision, may be carried out in 15-20 min., and is applicable to a wide range of products. For the determination of water in organic solvents the production of opalescence when added to carbon disulphide or light petroleum may be used as a limit test. A further method which is occasionally of value is based on the lowering of the freezing point of phenol or a phenol-cresol mixture by the addition of water contained in the sample under test, and by this means 0.03 *per cent.* of water may be determined.

T. J. W.

Conductimetric Determination of Ash in Raw Sugars. T. R. GILLET (*Analyt. Chem.*,

1949, 21, 1084-1086). Experiments showed that a direct relationship exists between the specific conductivity of raw sugar solutions and their percentage sulphated ash. For Hawaiian raw sugar, this can be expressed as *per cent.* ash = $0.00160 \times$ specific conductivity. The conductivity of such solutions depends on the content of ionic matter (ash), the sugar being non-conducting. The determination is carried out in a dip-type cell with platinized gold electrodes, a 5 *per cent.* solution of the sugar is used, and a correction is made for the conductivity of the water by subtracting 73 *per cent.* of its specific conductivity before calculation of the result.

A. E. W.

Determination of Inorganic Phosphate (in Vegetable Products). J. B. MARTIN and D. M. DOTY (*Analyt. Chem.*, 1949, 21, 965-967). The authors have investigated the effect of variations in procedure in the Berenblum and Chain method for the determination of inorganic phosphorus and describe a modification which eliminates the defects of the original method. An aliquot portion of the usual aqueous extract of the material is transferred to a test-tube and after dilution with water a mixture of equal volumes of *isobutyl* alcohol and benzene is added, followed by solutions of sodium silicotungstate in dilute sulphuric acid to precipitate the protein and an ammonium molybdate reagent. The tube is closed with a cork and immediately shaken for 15 sec., and after subsequent separation of the two phases an aliquot portion of the *isobutyl* alcohol-benzene solution is removed, diluted with an alcoholic solution of sulphuric acid and treated with a dilute stannous chloride solution. A blank prepared with the reagents only is treated in a similar manner and the percentage transmittency of the test liquid is determined in a photometer adjusted to read 100 *per cent.* with the blank using a waveband between 625 and 725 $m\mu$. The amount of phosphate present is ascertained from a standard curve correlating the percentage transmittency with definite known amounts of a phosphate solution. The blue colour finally obtained is stable for at least 24 hr., provided that the benzene and alcohol used are free from impurities.

T. J. W.

Estimation of Aluminium in Beer. J. W. TULLO, W. J. STRINGER and G. A. F. HARRISON (*Analyst*, 1949, 74, 296-299). The method described entails measurement of the fluorescence of the aluminium as 8-hydroxyquinolate. The procedure is as follows: 25 c.c. of the beer are evaporated to a syrup in a silica Kjeldahl flask and the organic matter destroyed by wet combustion with nitric and sulphuric acids (prepared by distillation from Pyrex glass and silica stills respectively). The last traces of nitric acid

are removed by adding about 0.5 gm. of ammonium sulphate to the cool, colourless sulphuric acid residue, washing it in with distilled water and heating the acid to fuming again for 5 min. The acid is diluted with about 40 c.c. of water, cooled and transferred to a 100 c.c. or 150 c.c. separating funnel, 10 c.c. of 0.2 per cent. 8-hydroxyquinoline in chloroform added and, after a few sec. shaking, ammonia to bring the p_H between 8 and 11. The funnel is then vigorously shaken for 30 sec., the chloroform layer allowed to settle and run off into a 20 c.c. flask, and the aqueous layer extracted with two 4-c.c. lots of chloroform, which are run off into the 20 c.c. flask and the volume adjusted to 20 c.c. with chloroform. This extract is clarified with about 1 gm of anhydrous sodium sulphate, and diluted either 4-fold (if light in colour) or 10- or 20-fold (if the colour is dark, due to the presence of iron or other 8-hydroxyquinolinates) with chloroform. Dilution of at least 4 times is necessary to avoid absorption of ultra-violet light by the reagent. The diluted extract is placed in a Spekker fluorimeter, using a Hilger No. 5 (green) filter in front of the measuring photocell, and balanced against the chloroform solution with the drum set at zero. A solution containing 200 microgm. of fluorescein per 150 c.c. of water is next placed in position, and the drum rotated to give a balance. The value of the antilog of $(2 - R)$ is calculated (R = drum reading), and from this value the amount of aluminium in the extract may be found by reference to a standard curve. Standard curves are made by treating from 0 to 80 microgm. of aluminium in the same way as in the test, and determining their fluorescence intensity against the standard fluorescein solution as above. It has not so far been found necessary to remove iron or other elements from beer, and the ratio of iron to aluminium present may exceed 10 to 1 without causing interference. Good recoveries have been obtained for aluminium added to beer, and duplicate analyses have agreed well. The method appears to be specific for aluminium and will measure as little as 0.01 parts per million in beer.

A. A. D. C.

Molybdenum Determination in Plant Material. Modification of Thiocyanate Stannous Chloride Method. I. BARSHAD (*Analyt. Chem.*, 1949, **21**, 1148-1150). The method is based on the formation of an amber to orange-red coloured compound when stannous chloride is added to a molybdate in the presence of thiocyanate. The colour is intensified by traces of iron and stabilized by nitrate. The plant material is ashed and the ash dissolved in hydrochloric acid. A little ferric chloride is added if no iron is present, followed by sodium

nitrate and potassium thiocyanate. Finally, a solution of stannous chloride is added and after making up to volume the colour is read in a colorimeter using a green filter. By this method, barley was found to contain 18.8 parts per million of molybdenum.

A. E. W.

II.—FERMENTATION

Riboflavin Production by Yeasts. H. LEVINE, J. E. OYAAS, L. WASSERMAN, J. C. HOOGERHEIDE and R. M. STERN (*Industr. Engng. Chem.*, 1949, **41**, 1665-1668). Detailed laboratory and pilot plant studies have been made of the production of riboflavin by the yeasts *Candida guilliermondia* and *C. flaveri* for the purpose of reducing the cost of manufacture. The procedure used consisted of shaking the inoculated medium in flasks for 4 or 5 days at 30° C., after which the yeast was removed by centrifuging and the riboflavin content of the liquid determined by a fluorimetric method which was frequently checked by a microbiological assay. The only sources of nitrogen necessary are ammonium sulphate and/or urea if the iron content of the medium is low, and the addition of asparagine or glycine is unnecessary. No adverse effect was produced on the yield of riboflavin by considerable amounts of manganese, copper, zinc, tin, nickel or aluminium but inhibition was produced by iron in amounts exceeding 50 microgm. per litre. Commercial grades of most chemicals, etc., used in the medium may be substituted for the pure substances without any deleterious effect and sterilization of the medium was found to be unnecessary. Urea and ammonium sulphate in proportions containing the same weight of nitrogen were equally efficient except that with *C. flaveri* ammonium sulphate led to a reduced yield. Also with this yeast sterilization of the medium gave low results unless asparagine or glycine were added. With both yeasts an increase in the amount of ammonium sulphate was without effect but the amount of riboflavin produced was roughly proportional to the amount of sugar added up to 6 per cent. Different lots of both glucose and sucrose were found to give various yields but the cause of these discrepancies was not ascertained. A number of pilot plant productions each using 24 litres of medium were carried out and an average yield of 191 microgm. of riboflavin per ml. was obtained, but in some experiments *C. flaveri* was found to produce sub-strains which failed to give riboflavin, although this was overcome by modifying the procedure.

T. J. W.

Influence of the Amino Acid-Dextrose Reaction on Growth of Lactic Acid Bacteria. D. ROSE and R. PETERSON (*Canad.*

J. Res., 1949, 27B, 428-436). Glucose-containing media darken in colour when heated, due to the reaction (Maillard) between amino acids and reducing sugar. If the darkening is intense, an inhibitory effect is often obtained with some micro-organisms. To determine the effect of Maillard products on the organisms used in microbiological assay, their growth has been examined in synthetic medium and in casein hydrolysate medium. The organisms used were *Lactobacillus arabinosus*, *L. casei* and *Streptococcus faecalis*, and the added Maillard product was prepared by heating casein hydrolysate with glucose for several days. If the glucose was autoclaved separately, and then added to the medium, growth of *L. arabinosus* and *L. casei* suffered a prolonged lag phase which was even more evident when a light inoculum was used. *S. faecalis* was much less sensitive to the factors than were the lactobacilli. Addition of preformed Maillard product to medium in which glucose was added after autoclaving gave little enhanced growth and the presence of a secondary factor in autoclaved glucose-containing medium was indicated. Careful control of p_H showed that this was not a critical factor, and although the addition of ascorbic acid enhanced growth, the oxidation-reduction potential was greatly different from that of any of the media produced without it; oxidation-reduction potential therefore does not appear to be critical.

During the Maillard reaction the glucose reacts with an amino acid, which, if essential and limited in quantity, leads to a lowered growth response. The authors have demonstrated that tryptophane was destroyed when tryptophane-assay medium was autoclaved. A serious error may thus be introduced into microbiological assays for tryptophane if glucose (or other aldehyde) is present before autoclaving.

A. E. W.

Influence of Magnesium on Cell Division.

II. Effect of Magnesium on the Growth and Cell Division of Various Bacterial Species in Complex Media. M. WEBB (*J. gen. Microbiol.*, 1949, 3, 410-417). Seven species of the genus *Bacillus* and two species of *Clostridium* grown in magnesium-deficient peptone media, formed long chains and filaments, indicating the essential nature of magnesium for normal growth. Temperature had a profound effect on filament formation, and an excess of magnesium had an effect similar to deficiency. *Kurthia zenkeri* and two species of *Lactobacillus* likewise formed filaments with excess or deficiency of magnesium. No filaments were formed with eight strains of Gram-positive and Gram-negative cocci but magnesium was essential for growth. Nine strains

of Gram-negative rods with magnesium deficiency grew better than the Gram-positive rods but required less excess magnesium to produce filamentous growth. It is concluded that the Gram-positive bacteria require more magnesium than Gram-negative species because the former utilize magnesium in the formation of the Gram-complex which is known to contain this element.

A. E. W.

Influence of Magnesium on Cell Division.

III. Effect of Magnesium on the Growth of Bacteria in Simple Chemically-defined Media. M. WEBB (*J. gen. Microbiol.*, 1949, 3, 418-423). In a simple chemically-defined medium, 15 bacteria failed to grow in the absence of magnesium. The Gram-negative bacteria gave maximum growth in the presence of 2-4 p.p.m. of magnesium, but Gram-positive species required 20-40 p.p.m. for maximum growth. The difference is attributed to the incorporation of magnesium in the Gram-complex. Since no growth takes place in the complete absence of magnesium, the element appears to be essential for the synthesis of bacterial protoplasm; it is also necessary for cell division (preceding abstract). In chemically-defined media, sub-optimal concentrations of magnesium do not give filamentous growth and it appears that in such media, the magnesium is sufficient for normal cell division of the limited population which the medium is able to support.

A. E. W.

Nitrogen Utilization and Growth of Coliform Bacteria. I. Adaptation to Growth in Ammonium Salt Media. G. A. MORRISON and C. HINSHELWOOD (*J. chem. Soc.*, 1949, 372-375). Three strains of *Bact. coli* grew readily in glucose-phosphate-asparagine medium but in glucose-phosphate-ammonium sulphate medium a long initial lag was required. The lag was progressively reduced by serial passage through the asparagine medium before transferring to the ammonium salt medium. Since asparagine media undoubtedly produce ammonia this is equivalent to a training process. When the organism grew in an alanine medium before transferring to the ammonia medium, the lag in the latter was dependent on the time allowed for the cells to remain at rest in the alanine after growth. Elimination of the long lag does not complete the adaptation, since further subcultures are required to secure optimum growth rate during the logarithmic phase. The training is distributed over the whole bulk of the bacterial population, showing that the phenomenon is one of adaptation rather than one of spontaneous mutation.

A. E. W.

Nitrogen Utilization and Growth of Coliform Bacteria. III. Nitrogen Utilization and the Lag Phase. G. A. MORRISON and C. HINSHELWOOD (*J. chem. Soc.*, 1949, 380-384). A glucose-phosphate-magnesium sulphate medium containing ammonium sulphate was inoculated with a strain of *Bact. coli*. No ammonia was utilized until growth commenced after a lag of 350 min. Two similar media, complete except for glucose and ammonium sulphate respectively, were inoculated. After measured times, the media were completed and the lag determined. In the medium containing glucose, the lag is constant from the time of inoculation; in the medium omitting glucose, the lag is constant from time of completion. Traces of glutamic and aspartic acids were detected just before growth commenced. If the organism is trained in asparagine and during growth transferred to media with other nitrogen sources, the lag is zero when the parent culture is growing actively. As the parent culture aged, the lag increased most rapidly in the medium containing only ammonium sulphate and least rapidly in the medium containing both glutamic and aspartic acids. The rate of growth in media containing these amino acids is less than in those containing ammonium sulphate or asparagine. It therefore appears that aspartic acid may initiate growth by a mechanism which is not the optimum one. To determine whether the amino acids or their carbon skeletons were responsible for the reduction in lag, experiments were carried out with the corresponding keto acids, oxaloacetic acid and α -ketoglutaric acid. The latter were effective, particularly α -ketoglutaric acid, but not so effective as glutamic acid, which appears to play a key rôle. A. E. W.

III.—BEER

Bottled Beers of Long Life. E. MOORTGAT (*Bull. Assoc. anc. Etud., Louvain*, 1949, 45, 65-79). By "long life" the author means a life of at least 2-3 months at ordinary temperatures. In theory there are three possible ways of preventing microbiological changes in a finished beer: by making the beer of such a nature that it will not support the growth of yeasts or bacteria, by killing the micro-organisms already present and preventing the entry of others, or by removing the micro-organisms instead of killing them. Various attempts to make beer resistant to yeasts and bacteria by reducing its content of carbohydrate or nitrogenous matter to a minimum, by adjusting its p_H to a low level, by adding small amounts of chlorine or penicillin, or by the oligodynamic action of silver, have been unsuccessful, and the use of preservatives is both objectionable and prohibited. Attempts to kill the micro-organisms by

the use of X-rays, ultra-violet rays, and electronic methods have also had no success, and heat remains the only practicable agent. Pasteurization in bulk followed by aseptic bottling is giving way to pasteurization in bottle, which is simpler and more certain. It should be remembered, however, that the effective pasteurization temperature varies with the p_H and alcohol content of the beer, and that cleanliness at all stages of brewing and bottling is as essential for a beer destined for pasteurization as for a beer which is not. Micro-organisms can be removed from beer by centrifuging or sterile filtration; the first method is capable of only a very low output, and does not improve the colloidal stability of a beer as does sterile filtration. The latter is an excellent system provided aseptic conditions can be assured beyond the filter. The filter itself, the mains, the bottling tank (to be eliminated if possible) and the bottling machine can be sterilized by steam, after cleaning, but it is less easy to ensure sterile bottles. These should be washed at 75°-80° C. (167°-176° F.) with a 1 per cent. solution of a detergent possessing adequate cleansing, sterilizing and rinsing qualities (a mixture of 25, 70 and 5 per cent. of trisodium phosphate, caustic soda and a sulphonate respectively is recommended), and as an added safeguard the bottles may either be filled with sulphur dioxide before bottling, or an antiseptic such as a quaternary ammonium compound may be added to the rinsing bath. Care must be taken that the antiseptic has no effect on the taste, odour or colloidal stability of the beer. The bottle caps can be sterilized either by flaming or by treatment with trioxymethylene vapour or sulphur dioxide, and air infection may be minimized by providing a cover above the line of bottles between the filling and capping machines, the underside of the cover bearing tubular ultra-violet lamps. The importance of preventing oxidation where beer is to be pasteurized is discussed, and the relative merits of pasteurization and sterile filtration are summarized. Pasteurization has the advantage of complete microbiological security, and the disadvantages of possible colloidal haze and a slight alteration in flavour which is almost unavoidable. Sterile filtration has the advantage of not altering the flavour of the beer, but the maintenance of asepsis in the bottling plant is difficult and microbiological security is less certain.

A. A. D. C.

p_H and r_H in Brewing. E. URION (*Brasserie*, 1949, 3, 140-147). The p_H factor exerts an influence at every stage of brewing. In the mash tun it affects saccharification and proteolysis, in the copper it affects the type and extent of extraction of the hops, the formation of colour, and the "break," in the fermenting

vessel it affects the degree of attenuation, and in the beer it is related to the appearance of colloidal and biological hazes. Experience has shown that for a pale ale of 1040–1048° sp.gr. the p_H should lie between 5.8 and 6.0 in the mash, between 5.2 and 5.4 at the end of the boil, and between 4.2 and 4.4 in the finished beer. The r_H factor is equally involved. It expresses the degree of oxidation or reduction of a solution, and can range from 0 to 42. The lower the r_H the more highly reduced is the liquid. In general, values below 20–25 indicate a reduced state, and values above that an oxidized state. The r_H of wort influences colour-formation, the transformation of the hop resins, and the precipitation of tannin-protein compounds in the copper. A limited amount of oxygen in solution is needed for yeast multiplication. The exact requirements are unknown, nor is it known whether the oxygen may be chemically combined or must be free, and in the author's opinion many brewers aerate their worts more than is necessary. At the end of the primary fermentation the r_H of the beer is about 9, and in storage tank it is generally between 9 and 11. During racking or bottling it may rise as high as 20 for a short time, and then falls slowly to a value which depends on the amount of air which has entered and the quantity of reducing bodies in the beer. Two kinds of substances are involved in these changes. The initial rapid rise in r_H is brought about by oxygen-carriers in the beer (notably iron and copper ions), and the subsequent slow reduction is due to bodies of the melanoidin or reductone type which, by reason of their dienol-diacetone equilibrium, oppose undue rise or fall in r_H , as buffer substances oppose undue change in p_H . These reducing bodies are derived principally from the malt, but some may be formed during wort boiling, and some added as caramel. Increase in the r_H of beer favours the development of yeasts, the formation of oxidation haze, and the production of a "cooked" flavour in pasteurized beers. An r_H below 13 favours the formation of "sun flavour," but this is avoided by the use of amber glass bottles. A. A. D. C.

Reductones in Brewing. Influence of Malt on the r_H —Poise of Wort and Beer. E. SCHILD and M. V. HUGMANN (*Brewers' Digest*, June, 1949, 43–47). Reductones are substances which reduce Tillman's reagent and which are without antiscorbutic activity; their properties depend on an enol group and adjacent double bond or upon a dienol grouping. The melanoidins are related to the reductones and also have reducing properties; both are formed when sugars are boiled, the former only if nitrogenous matter is present. Both compounds are formed when wort is boiled and their

reducing nature assists in maintaining a low r_H in the finished beer, with a resultant increase in both biological and non-biological stability. In this respect, dark beers are better than pale beers, and unfortunately any increase in reductones is at present always accompanied by an increase in colour. Prolonged boiling is at present the only known method of increasing reductones in the brew-house but the present experiments show that the reducing character of the wort is largely dependent on the malt. Special r_H patent malt has a high acid content but affords a well-buffered wort of p_H 5.46, which has a high reducing activity when measured by the Indicator Time Test. The test showed that the reducing value of wort from normal malt increased progressively during boiling, but the reducing value of wort from r_H malt increased to an even greater extent. When the two worts were exposed to the atmosphere, oxidation of the normal wort took place rapidly, but oxidation of r_H wort was extremely slow. The reducing power of wort may be conveniently expressed by the function $10,000 \times \text{reciprocal I.T.T. value}$. In this way, normal wort as mashed has only one-half the reducing value of r_H wort, after 2 hr. boiling $1/110$ and after exposure of the wort to the atmosphere for 72 hr. $1/70$. Reduction values of beers prepared from these worts have not been determined, but the experiments afford an overall insight into the oxidation stability of the materials examined. A. E. W.

Air and Bottled Beer. M. G. VAN ROEY (*Bull. Assoc. anc. Etud., Louvain*, 1949, 45, 53–64). The air in a bottle of beer is in two forms: as a gas in the free space, and dissolved in the beer. The solubility of air in beer is 21 c.c. per litre at 10° C. (50° F.) and, since oxygen is slightly more soluble than nitrogen, one-third of this amount, or 7 c.c., will be oxygen. The dissolved oxygen is slowly absorbed by reducing bodies in the beer, and more oxygen dissolves from the gas in the free space to take its place. This absorption of oxygen produces changes in the beer. After pasteurization, it gives rise to the unpleasant "cooked" flavour, which is due to a volatile substance, possibly an ester. The beer darkens, owing to oxidation of the tannins. Should it be exposed to sunlight, the presence of the oxygen will retard or prevent the appearance of "sun-flavour," by oxidation of the sulphydryl groups which would otherwise give rise to the mercaptans and hydrogen sulphide causing the flavour. This oxidation at the same time makes the proteins to which the sulphydryl groups belong less soluble, and so leads to haze formation. Other proteins are similarly affected, particularly through the agency of iron and

copper ions, which act as oxygen carriers, and oxidation of tannins produces insoluble phlobaphenes. The rise in r_H caused by the oxygen enables yeast to develop anew in the beer, and oxidation of other constituents changes its flavour and aroma. Information about oxygen in bottled beer can be gained in three ways: by direct determination of the free and dissolved air, by determining the r_H of the beer, and by applying to it the Indicator Time Test (I.T.T.). The author gives a brief description of the methods used and quotes results of his own and other workers, from which it appears that there can be considerable variation in the amounts of both free and dissolved air in different bottles of the same bottling, and that the I.T.T. is affected to a marked degree by the temperature, the presence of reductones, and the presence of iron. Precautions against oxidation of beer must start as soon as fermentation is ended, and be applied at every stage of handling. The natural reductones should be conserved; others may be added immediately before bottling if desired. The air in the free space can be kept to a minimum by causing the freshly bottled beer to fob before it is capped. A. A. D. C.

Determination of Air in Bottled Beer.

E. URION and G. LEJEUNE (*Brasserie*, 1949, 3, 148-150). The method described can be applied to bottles with any type of closure. The liquid and gas enclosed in the bottle are brought to a state of equilibrium and the air in the free space determined, from which can be calculated the dissolved air. The procedure is as follows: The bottle of beer is kept in a water-bath at a temperature of about 10° C. (50° F.) for several hours with frequent inversion and shaking. The level of the beer is marked on the neck of the bottle, which is then opened (while still submerged) under an inverted funnel connected by rubber tubing to a Bunte burette, the funnel and tubing being full of water. The gas from the free space in the bottle is aspirated into the burette, the carbon dioxide absorbed in caustic soda solution, and the volume of the remaining air measured at atmospheric pressure. The bottle is removed full of water from the bath, the closure replaced and then removed, the volume of the liquid down to the mark on the neck measured (free space), and finally the volume remaining in the bottle (beer volume). Since the solubility of air in beer is approximately 90 per cent. of its solubility in water, the volume of air dissolved in the beer (Ab) will, by the law of partial pressure, be $0.009 St.V.Ag/v$, where St is the number of c.c. of air dissolved by 100 c.c. of water at atmospheric pressure and temperature T , at which the measurements were made, V is the volume of the beer, Ag is the determined volume of air

in the free space, and v is the volume of the free space. The total air will then be the sum of the free and dissolved air volumes. The accuracy of the method has been tested by attaching tested bottles of beer to the Bunte burette and driving all dissolved gas into the burette by heating the beer to boiling point. The figures for dissolved air obtained by this means have not differed by more than 0.5 c.c. from those calculated by the above method. This accuracy is considered sufficient for purposes of routine control, but it is emphasized that to get this accuracy the conditions ensuring equilibrium between free and dissolved gas in the bottle must be observed. The method has been used to verify the claim that causing freshly bottled beer to fob by a shake of the bottle between filling and capping reduces the air in the free space to a minimum. Determinations of air in the free space immediately after bottling, with no attainment of equilibrium between free and dissolved gas, have been used for examining the efficiency of the different jets of a filler-head. A. A. D. C.

IV.—PLANT, MACHINERY AND BY-PRODUCTS

Balsa Wood as Insulation in Breweries.

J. H. BERGMANN (*Amer. Brewer*, Aug., 1949, 19-21, 46-48). Owing to difficulty in obtaining any of the usual materials, Balsa wood was used as an insulating medium for the internal walls of the fermentation cellar in a small Central American brewery and the author describes the characteristics of this timber and the methods used in its application. The lightness of the wood is due to the large volume of air contained in its cells and the almost complete absence of the relatively heavy lignin from the cell walls, and when in a dry condition its heat conductivity is very low. Since balsa readily absorbs moisture it was waterproofed before use by kiln drying and dipping each board into heated asphalt. The old brick wall was bored and fitted with wooden pegs to act as supports, then coated with layers of asphalt; strips of bitter cedar (a wood which is not attacked by termites) were nailed to the pegs. Finally, balsa boards 4 in. thick were fitted to the exterior surface and others of half this thickness were applied on the interior, both being coated with a layer of cement held in position by wire netting and projecting nails. When the cellar was in use no difficulty was experienced in maintaining the temperature at 43° F. and during two days when the brine pump was inoperative a rise of 4° only was observed, although several tanks contained beer some of which was in active fermentation. The success obtained with this cellar induced the owners to thus insulate the whole refrigerating area of the brewery and in order to prevent attack by

termite the balsa wood before use was sprayed at frequent intervals with 10 *per cent.* Montanin solution. A similar procedure with some modifications was used in its application, but instead of wire netting for retaining the plaster the outer layer of wood was scored and grooved. It is suggested that a layer of balsa wood should be incorporated as an insulating layer in the "Sandwich" construction consisting of layers of wood or metal for the production of casks. Although balsa and cork have several useful qualities in common, the wood is of less value since it is not vermin- or fire-proof, and unless waterproofed readily absorbs moisture which reduces its efficiency as an insulator. T. J. W.

Use of Kieselguhr Filters in the Brewery.

A. MOLL (*Schweiz. Brau.-Runds.*, 1949, 60, 143-145). Observations were made on the use of kieselguhr for filtration of worts and beers in the brewery. On filtering hot worts through kieselguhr, brilliant filtrates were obtained, but a fine turbidity came down in the coolers and fermentation proceeded more slowly than normal. Filtration of the worts after cooling to 5° C. was more satisfactory. Such worts came through brilliant, fermented more rapidly than normal and produced a fine white yeast crop. The beers matured for drinking in 10 days and showed improved head-retention over normally processed beers. The process of cold wort filtration through kieselguhr was especially suitable for Pilsner-type beers in closed fermentations with CO₂ recovery, but not for dark beers. The filtration of beer as usually carried out through kieselguhr supported on cloth or mesh, suffered from three disadvantages: (1) susceptibility to mechanical shock; (2) the need for relatively large quantities of kieselguhr; (3) the considerable time taken before the beer ran bright. These difficulties were overcome by depositing the kieselguhr on hard filter discs. The quantity and kind of kieselguhr used depended on the beer, varying between 30-50 grm. *per* hectolitre. For 200 hl. of beer, 2.5-3 kgrm. of kieselguhr were deposited on the disc, the remainder being added as filtration proceeded. 20-25 hl. of beer *per* hr. were filtered through a filter face of 5 sq. metres, the filter passing 160-220 hl. before a change of kieselguhr was necessary. The kieselguhr imparted no flavour to the beers, which were deemed to have a fine flavour and a full palate. They showed an improved head-retention and biological stability and were less chill-sensitive than normally processed beers. Filtration losses were reduced, the overall cost of filtering 200 hl. of beer being Fr. 12, compared with Fr. 34 in the case of a pulp filter. C. R.

The Beer Bottle. P.-A. CARON (*Brasserie*, 1949, 3, 170-175, 190-199). This is an account

of work done on behalf of the Association for the Study and Control of Materials used in Brewing and Malting (France). As a result of the work, specifications for beer bottles have been suggested and certain types have been standardized. The study is comprehensive and contains much factual information about: (1) glass and the manufacture of bottles; (2) the general characteristics of a beer bottle; (3) types of bottle tops; (4) the colour of bottles; and (5) the resistance of bottles to temperature and pressure. In the first section are given the composition of several kinds of glass and the materials used in their manufacture, followed by an outline of the process of making bottles. The second section contains information and recommendations concerning shape, capacity, dimensions and weight, and in the third section the design, dimensions and faults of bottle tops are discussed, with emphasis on the type taking crown corks. In dealing with the colour of the bottles, in section four, the author reviews the work that has been done on the influences of light on beer flavour, which has shown amber glass to be the most satisfactory. In the final section the effects on the beer bottle of the changes of temperature and pressure encountered during washing, filling, capping, pasteurization and storage are examined. These effects have been studied by measuring the resistance of the bottle to: momentary pressure, continuous pressure, thermal shock and pressure and thermal shock combined. The theory of the forces and strains set up during pasteurization (pressure and thermal shock, combined) is briefly set out, and the results that have been obtained from each type of test are recorded. For many details of the recommended specifications the original report must be consulted, but the standards of durability can be stated briefly. The bottles should withstand transferring from cold water to water at least 35° C. (63° F.) hotter, and *vice versa*, without breakage, and they should withstand a pasteurization test with not more than 2 *per cent.* breakages. The pasteurization test consists in filling the bottles at a specified pressure and with a specified free space (depending on the size of the bottle), raising their temperature from 10° to 68° C. (50° to 154.4° F.) in 20 min., holding at 68° C. for 30 min., and cooling them from 68° to 10° C. in 20 min. A test equivalent to this is to submit the bottles to an internal pressure of 5 kgrm. *per* sq. cm. (71 lb. *per* sq. in.) at a temperature of 68° C.

A. A. D. C.

Use of Sterilizing Quaternary Ammonium in the Bottling Store. J. C. L. RESUGGAN (*Bottling*, Oct., 1949, 46-50). Quaternary ammonium compounds have recently been applied to the sterilization of pipelines, fillers

and other equipment in the bottling stores, but they will not necessarily supersede older reagents. A study of the physical and chemical properties of quaternary salts will indicate the best methods of using them as well as their limitations. These compounds have a very strong affinity for wood, more especially if used in conjunction with other surface-active agents. Their effect on wood is cumulative and thus the wood may become contaminated with a preponderantly Gram-negative micro-flora. In addition, their surface-active properties may release substances imparting a sticky surface to the wood. If the wood is already impregnated with acidic substances, the p_H of the quaternary solution will be lowered and its effect on certain micro-organisms thereby altered.

Although generalization is not always possible with this group of compounds, the twin chain type are usually less affected by anionic reagents and other organic compounds than are salts of the single long chain type. Thus the single long chain type often flocculate with anionic reagents, hard water, calgon, *etc.*, but with the twin chain types a cloudiness is usually the only effect noticed on addition of these substances. The twin chain type of compound used in many breweries is non-corrosive but may impart a stickiness to some types of synthetic rubber, an effect which can be removed by a mildly alkaline wash. The compound imparts a negligible taste if rinsing of the plant is thorough. It is marketed as a liquid which does not crystallize, and solutions of it may be used several times before discarding. There are indications that the twin chain type of compound is less adsorptive than the long chain type and although it is important to see that surfaces are reasonably clean before sterilizing, the compounds deal with considerable quantities of organic matter and the preliminary cleansing of plant is therefore of less consequence than usual.

Recent work with four different quaternary ammonium compounds has indicated that the particular salts tested were non-toxic at concentrations much greater than any likely to be accidentally present in a beverage. The highly penetrating nature of the solutions render them effective in sterilizing valve seatings, crevices and bottle stoppers. The solutions generally used vary from $\frac{1}{2}$ to 2 c.c. of quaternary salt *per* gallon as working concentrations.

A. E. W.

Disposal of Effluents Containing Synthetic Detergents. H. H. GOLDTHORPE, W. H. HILLIER, C. LUMB and A. S. C. LAWRENCE (*Chem. & Ind.*, 1949, 679-682). The chief objects of sewage treatment are to remove

settleable solids, prevent the silting up of water-courses and eliminate organic matter so that the oxygen absorbed will not impair the life of vegetable and animal organisms when the effluent is discharged into a stream. The quality and quantity of the polluting matter in sewage are continually varying, and in general two processes are used for its purification—sedimentation, which may be facilitated by the addition of lime or other chemicals and removes the larger solid particles, and the subsequent biological action of bacteria, *etc.*, eliminates the suspended, colloidal and soluble impurities. Owing to the shortage of fats in recent years, soap has been largely displaced by synthetic detergents such as Teepol, Lissapol N, *etc.*, and since sewage works procedure has been largely based upon the presence of soap some anxiety is aroused by the substitution of the newer detergents. These synthetic products, unlike soaps, are unaffected by the coagulants used and tend to prevent the clumping essential to satisfactory sedimentation. Thus solids, which should be eliminated in the preliminary treatment, are carried forward into the biological system together with grease and oil which have been emulsified and cause extreme difficulty in de-watering and the removal of grease. Little is yet known about the effect of synthetic detergents on the activities of organisms in the percolating bed or the activated sludge process, but experiments indicate that the elimination of oxygen-absorbing substances is less efficient than when these detergents are absent; considerable extension of biological plants may be required to compensate for the reduced efficiency. At present only two of these detergents are in general use, but many others are in production and it is probable that each will require different treatment to eliminate its adverse effects on the purification of sewage. In the discussion following this paper widely divergent views were expressed upon the effect of synthetic detergents in sewage, and it is evident that owing to the number of factors concerned much more work is necessary before definite conclusions can be reached. T. J. W.

V.—BIOCHEMISTRY

Occurrence and Significance of the Pentose Sugars in Nature, and their Relationship to the Hexoses. E. L. HIRST (*J. chem. Soc.*, 1949, 522-533). The pentoses xylose, lyxose, ribose and arabinose and the methylpentoses rhamnose and fucose are of historical interest because of the part they played in the elucidation of the stereochemistry of the hexoses, but more recently it has been evident that pentoses are quite widely distributed in nature. D-ribose and its 2-deoxy derivative are the carbohydrate components of the nucleic acids, and pentoses also occur in

complex polysaccharides which act as food reserves in plant seeds and in the cell walls of plants.

There are stereochemical relationships with the hexoses. D-xylose is similar to D-glucose and L-arabinose similar to D-galactose; in both cases the hexose can be converted to the pentose by oxidation followed by decarboxylation and it is interesting to observe that D-galacturonic acid, D-galactose and L-arabinose on the one hand or D-glucuronic acid, D-glucose and D-xylose on the other, are often found associated in plant gums and mucilages. In nature, the pentoses may therefore arise by a mechanism of this type but such transformations could not take place at polysaccharide level and would in this case be involved in a preliminary breakdown followed by synthesis of new structures. The pentose D-ribose occurs in riboflavin, coenzyme I and in nucleic acids. Arabinose residues are involved in the structure of araban which is associated with the pectic material of many plants and xylose is contained in xylan, the wood gum occurring with cellulose in woody plants. More frequently, however, the polysaccharides contain more than one of the pentoses. The problems of the structure of the gums are therefore manifold but nevertheless are slowly being resolved. On the whole D-galacturonic acid tends to be a characteristic of the plant gums, often together with D-galactose and L-arabinose; some gums, however, contain no pentose and in others methylpentose (e.g., L-rhamnose) replaces the pentose wholly or in part. Usually only one of the optical isomerides of each sugar is detectable, for example, L-arabinose is usually found, but exceptions sometimes occur and the polysaccharide from *Mycobacterium tuberculosis* contains D-arabinose. In general, D-xylose occurs in the pyranose form and is usually linked to another reducing sugar: arabinose usually adopts the furanose structure, occurs as the D and L forms (as does the related hexose galactose), and is generally found in the outer parts of the gum molecules. Xylose (and the related hexose, glucose) occur only as the D-forms. Nothing is known of the nature of enzymic processes leading to polysaccharide synthesis from pentoses but it would not be surprising if mechanisms involving phosphorylases were discovered to be responsible. The plant gums are constant in their composition and chromatographic analysis of their components may suffice to identify their botanical origin. In the plant they often result from a stimulus due to injury and may be associated with the disappearance of the reserve starch.

A. E. W.

Thermal Disruption of Starch Grains.
R. BREBANT (*Indust. Agric. Aliment.*, 1949,

141-153). The effect of temperature on various starches was studied by passage of a starch paste between two heated plates, the paste being then examined microscopically. The water absorptions of the treated and untreated starches were measured by a simple decantation method. The bursting and water absorption of starch are functions of its chemical composition, starches with an ash content of less than 0.3 per cent. show a large proportion of burst grains and high water absorption at 115-120° C., whereas high-ash starches require a much higher temperature (130-145° C.) for the same effect. The water absorption and bursting are inversely proportional to the content of calcium, phosphorus and lipids. Amylose is expelled from burst granules and is not associated with any of the non-saccharide constituents of the grains. Some of the non-saccharide constituents are associated with the amylopectin portion of the starch. A. E. W.

Separation and Determination of Methylated Aldoses. E. L. HIRST, L. HOUGH and J. K. N. JONES (*J. chem. Soc.*, 1949, 928-933). Filter paper chromatography permits the separation of closely-related methylated sugar derivatives and provides a procedure for the rapid identification of such derivatives from hydrolysates of methylated polysaccharides. 2 : 3 : 4 : 6-tetramethyl D-glucose was used as reference standard, the ratio between the distance the unknown substance moved and the distance the standard moved, giving a constant (R_F), characteristic of the sugar. The apparatus and solvents used were those previously described (*J. chem. Soc.*, 1948, 1679). A small spot of test solution was placed on the starting line of the paper (15 × 50 cm.), alongside, and 3 cm. away from, a spot of 10 per cent. tetramethyl D-glucose. When the solvent was 40 cm. from the starting line, the paper was removed and dried. In qualitative experiments, the position of the separated sugars was shown by spraying with ammoniacal 10 per cent. silver nitrate. The hydrolysis of methylated polysaccharides submitted to this procedure was carried out by treating 50 mgrm. of the material with 1 ml. of 4 per cent. methanolic HCl in a sealed tube on the boiling water-bath. The tube was then opened and the contents subjected to hydrolysis with 4 per cent. aqueous HCl at 100° C. for 3 hr. After neutralization with silver carbonate and removal of silver by H_2S , acidic substances and uronic acids were removed by use of Amberlite Resin IR4B, and the neutral solution evaporated to a thin syrup, which was examined on the chromatogram.

The method was put on a quantitative basis by application of Sobotka's alkaline iodide method. To obtain measurable quantities

(0.5-3.0 mgrm.) of separated methylated aldoses, the method of Flood *et al.* (*J. chem. Soc.*, 1948, 1679) was employed, the filter strips thus obtained being extracted for 3-4 hr. with 5 ml. water, at boiling. After cooling to 20° C., the solution was oxidized in a stoppered tube for 2-2½ hr. with 1 ml. 0.1 N iodine solution in presence of 2 ml. of 0.2 M sodium carbonate-sodium bicarbonate buffer (p_H 10.6). After dilution to 25 ml. and acidification with 2 N H_2SO_4 , the liberated iodine was titrated with 0.01 N thio. The method had an accuracy of ± 8 per cent. and was satisfactory for glucose, galactose, arabinose, xylose, ribose and their methylated derivatives, but not for unmethylated rhamnose, lyxose and mannose. Applied to methylated animal glycogen and methylated waxy maize starch, all previously isolated methylated sugars were identified and estimated, together with other partly methylated sugars.

C. R.

Phosphorus Compounds of Wheat Starch. H. S. ROOKE, L. H. LAMPITT and E. M. JACKSON (*Biochem. J.*, 1949, 45, 231-236). Whereas in potato starch the phosphorus is present as an amylo-phosphoric ester, in wheat starch it is combined as phosphatide, loosely bound to carbohydrate (this *Journ.*, 1941, 392). The two wheat starches used containing about 0.07 per cent. of P, yielded little soluble phosphorus and nitrogen when extracted with ether and ethanol, so that peptization with chloral hydrate (this *Journ.*, 1949, 42) was carried out as a preliminary step. 9 gm. starch was dissolved by heating to 80° C. with 300 ml. of 33 per cent. (w/v) chloral hydrate solution, previously brought to p_H 7.0 with sodium bicarbonate, and maintained at 80° C. for 30 min. The starch was recovered by blowing the hot solution through a fine jet into 600 ml. dry acetone, pouring off and washing twice by decantation with 200 ml. portions of dry acetone. The aqueous residues after concentration of the acetone filtrates *in vacuo* contained 50-60 per cent. of the P in the original starch, and were used for five quantitative fractionations employing solvents. Samples of each fraction were taken for determination of N, P and choline. The bulk of the N and P were separated as one fraction, which was soluble in ether or chloroform, had a N : P ratio of 1 : 1, and in which almost all the N was present as choline. The phosphatide isolated from 86 gm. wheat starch was purified by dissolving in chloroform, precipitating with excess acetone and repeating three times. The product (50 mgrm.) was a white, waxy solid, agreeing closely in elemental composition with that calculated for oleopalmitophosphatide. The presence of phosphatide in wheat starch was therefore confirmed, the content being about

1 per cent. calculated from the choline content of the starch acetone filtrate. Of the P remaining in the starch precipitated by acetone after peptization, calculated on the P content of the original starch, 10 per cent. was extractable by 0.5 N HCl (inorganic and glycerophosphate), 30 per cent. remaining in association with carbohydrate. Hexose- and nucleotide-phosphate were absent and there was no conclusive evidence for phytic acid.

C. R.

Fractionation of Potato Starch by Means of Aluminium Hydroxide. E. J. BOURNE, G. H. DONNISON, S. PEAT and W. J. WHELAN (*J. chem. Soc.*, 1949, 1-5). Starch may be almost completely adsorbed from solution by aluminium hydroxide precipitated *in situ*. If less adsorbant is used, amylopectin is preferentially adsorbed. This method of fractionation, though less convenient for the routine isolation of amylopectin, proved useful for the separation of amyloses of high blue value. A 3 per cent. starch dispersion in 0.1 per cent. aqueous sodium chloride was boiled with stirring for 1 hr., cooled rapidly to 30° C. and a solution of 0.45 gm. aluminium nitrate ($9H_2O$) per 1 gm. dry starch added, followed by concentrated ammonia to permanent alkalinity to phenolphthalein. "Ageing" was allowed to proceed for 3 days at 30° C., both the temperature and time of ageing being important in obtaining amylose preparations with high blue values. After centrifuging off the aluminium hydroxide adsorbate, the supernatant was dialysed two days against running water and amylose precipitated from the dialysed solution with two volumes of alcohol. The precipitate was triturated with alcohol, then with ether, and dried. Yields (6-13 per cent.) were lower than those obtained by the usual methods, but blue values ranged from 1.35-1.40. Ash content was about 1 per cent. and contained aluminium and nitrate. That the method effects a true fractionation of starch was confirmed by light adsorption curves and by the fact that β -amylase yielded 91 and 89 per cent. maltose from amylose fractions having blue values respectively 1.39 and 1.37. C. R.

Amylose Component of Waxy Maize Starch. E. J. BOURNE and S. PEAT (*J. chem. Soc.*, 1949, 5-9). The proposition was examined that waxy cereal starches entirely lack the amylose component. The de-fatted waxy maize starch studied was free from ash and protein, was hydrolysed by acid completely to glucose, and the conversion limit with soya bean β -amylase was 49 per cent. The blue value of the starch was 0.10, compared with average values of 1.10 and 0.22 respectively for amylose and amylopectin fractions from potato starch. No amylose was obtained by use of amylose

precipitants, *e.g.*, thymol or cyclohexanol, except when these substances were used in the presence of N sulphuric acid, when fractions enriched in amylose were obtained. These fractions resembled potato amylose in staining properties (blue value 0.93-1.18) and maltose conversion limit (81-85 *per cent.*). Fractionation with aluminium hydroxide (see previous abstract) resulted in the separation of "amylose" fractions in yields of 0.7, 2.5 and 1.2 *per cent.*, having blue values 0.33, 0.26 and 0.40 respectively. The last fraction, having a limit of β -amylase conversion of 59 *per cent.*, thus resembled potato starch.

It is concluded that waxy maize starch does contain the unbranched amylose component, but the content is less than 2 *per cent.* The suggestion of Pacsu and Hiller that the amylose of waxy maize starch arises as the result of hydrolytic cleavage is thought unlikely, amylose being regarded as pre-formed in starches.

C. R.

Modified Method for End-group Assay of Amylose and Other Long-chain Starch Fractions. E. J. BOURNE, K. H. FANTES and S. PEAT (*J. chem. Soc.*, 1949, 1109-1113). Bell's partition chromatographic method (*J. chem. Soc.*, 1944, 473) was applied to measure the average chain lengths of amylose and amylopectin, and to find a reliable end-group assay method for 1:4- α -glucosidic polysaccharides applicable to small quantities (2-4 grm.) of amylose. The method consisted of four stages: (a) hydrolysis of the methylated polysaccharide; (b) removal of the acids; (c) extraction of an aqueous solution of the mixed sugars with chloroform and evaporation of the chloroform extract; (d) chromatography of a chloroform solution of the syrup from (c) on a silica-water column under conditions such that trimethyl glucose ("tri") was retained and tetramethyl glucose ("tetra") passed through. Methylation was carried out with sodium and methyl iodide in suspension in liquid ammonia at -70°C .

Methylated amylopectin gave satisfactory results by Bell's method, but assays of trimethyl amylose yielded syrups, instead of crystalline "tetra." Since partition of artificial mixtures of "tri" and "tetra" resulted in yields of crystalline "tetra," syrup formation took place during stages (c) and (b) of the procedure. The syrupy impurity was probably a pentamethyl disaccharide, arising by demethylation and "reversion" of "tri." Thus, in amylose assays, where the proportion of "tri" to "tetra" was high, the effect of the syrup was more marked. Bell's method was therefore modified by removal of "tri" before partition. The methylated polysaccharides were treated for 9 hr. under reflux with methanol containing 1.5-2 *per cent.* dry

HCl and the resulting methyl glucosides fractionally distilled at 0.01 mm. The first fractions, which contained all "tetra" and little "tri," were hydrolysed with 5 *per cent.* (w/v) aqueous HCl for 7 hr. at 100°C ., neutralized with silver carbonate and analysed for "tetra" by Bell's method. Chain lengths calculated from the analyses were in good agreement with those derived from classical end-group assay methods requiring much more material: amylopectin 18 ± 1 , potato starch 26 ± 1 , amylose (blue value 0.915) 101 ± 5 , amylose (blue value 1.210) 190 ± 10 .

C. R.

Enzymic Synthesis and Degradation of Starch. Role of Carbohydrate Activators.

E. J. BOURNE, D. A. SITCH and S. PEAT (*J. chem. Soc.*, 1949, 1448-1457). The nature and function of activators in the synthesis of amylose from glucose-1-phosphate by phosphorylase was studied. Activating powers were measured by a modification of the method of Green and Stumpf (this *Journ.*, 1942, 181) and were quoted relative to that of commercial soluble starch. Phosphorylase was prepared from potato juice purified by repeated precipitation with neutral ammonium sulphate, and was free from Q-enzyme, amylases and maltase.

The purest amylopectin was 2.9 times as active as the purest amylose, but a branched chain was not the essential feature of an active substance. Activating power seemed to primarily depend upon the number of chain-ends available *per unit weight* of activator. Further, the nature of the chain-ends was important, since limit dextrin-A, formed from amylopectin by β -amylolysis, had only one-tenth the activity of the parent amylopectin. This dextrin had the same number of chain-ends as amylopectin, but had lost the "outer" chains (up to the 1:6-linkages) through amylytic action. The "inner" chains were therefore less effective activators. The 1:6-glucosidic dextran synthesized by *Leuconostoc mesenteroides* had no power of activation, so that activity was also connected with α -1:4-glucosidic bonds. To be an activator, a carbohydrate had to be a polyglucose, in which were present terminated chains of glucose residues linked by α -1:4-bonds (template chains). Maltose was inactive, but amyloses, synthesized by phosphorylase, possessed activity in inverse proportion to chain length. Template chains therefore appeared to have both a minimum and an optimum length. Studies on the activating power of the products of acid hydrolysis and β -amylolysis of both amylose and amylopectin gave indications of these critical values. The initial fragmentation of amylose chains under the hydrolytic action of hot N sulphuric acid into shorter dextrin chains was accompanied

by an increase of activating power with little development of reducing power. Two maxima of activity were reached, before activating power diminished subsequently as hydrolysis to glucose proceeded. The corresponding hydrolysis of amylopectin also gave two maxima, but was distinguished by a slower rate of fall of activating power as hydrolysis proceeded. The presence of two maxima in both cases, was explained by the existence of an optimal template chain length, calculated to be of at least 20 glucose units, responsible for the first (minor) peak, and a minimum chain length of 5-6 glucose units responsible for the second (major) peak. The variation of activating power with chain length was demonstrated more clearly by hydrolysis with β -amylase, which acts by the successive removal of maltose units from the ends of the chains and not by fragmentation of the substrate into dextrans. With purified amylopectin, a rise of activating power took place in the early stages, followed by a general decrease to a low value when half of the substrate had been converted into maltose. This corresponded with the observation that limit dextrin-A had little activating power. With an amylose (containing some amylopectin), an initial fall in activating power as β -amylolysis proceeded was followed by a peak at 80 *per cent.* conversion, at which point the residual dextrans were calculated to be 15-25 glucose units, *i.e.*, in agreement with the optimum template chain length as deduced from the acid hydrolysis curve.

Studies on the phosphorolytic, as opposed to the synthetic, function of phosphorylase showed that amylose was readily converted into glucose-1-phosphate, provided inorganic phosphate was present. The processes of phosphorolysis and β -amylolysis of amylose were similar, both methods of attack splitting off single units from non-reducing chain-ends. α -Amylolysis followed a very different course. Amylopectin was partly degraded by phosphorylase in the presence of inorganic phosphate, the action of the enzyme being obstructed by the same linkages which it cannot synthesize, *i.e.*, the 1:6-cross linkages. However, phosphorolysis went to a limit of 39 *per cent.* conversion to glucose phosphate, whereas β -amylase hydrolysis (also impeded by the cross linkages) ceased at 53 *per cent.* conversion to maltose. This was reflected in the fact that limit dextrin-P, derived by phosphorolytic cleavage of amylopectin, was 6-7 times more potent as an activator in phosphorylation than limit dextrin-A. The outer (template) chains of dextrin-P were calculated to contain 5-6 units (this *Journ.*, 1944, 105). Dextrin-P was converted into dextrin-A by β -amylase. Neither the amylases, nor plant phosphorylase attack the 1:6-glucosidic linkages of amylopectin. C. R.

Purification and Storage of Q-Enzyme of the Potato. S. A. BARKER, E. J. BOURNE and S. PEAT (*J. chem. Soc.*, 1949, 1705-1711). Whereas phosphorylase catalyses the synthesis of amylose from glucose-1-phosphate, a second enzyme (Q-enzyme) is concerned in the synthesis *in vitro* of the amylopectin constituent of starch. The Q-enzyme has now been isolated and purified from the juice of King Edward potatoes. To the juice, adjusted to p_H 7.2, was added lead acetate solution (190 grm. *per* 3 litres) previously adjusted to p_H 7.25 with sodium hydroxide. The enzyme was precipitated with the lead-protein complex, which was removed at the centrifuge and eluted with 0.2 N sodium carbonate solution, through which a stream of CO_2 was passed. After discarding the residue, 50 *per cent.* ammonium sulphate solution (p_H 7.0) was added to the supernatant up to 19 *per cent.* final concentration. The precipitate was collected and redissolved in water (fraction Q 1). Reprecipitation of Q 1 with ammonium sulphate gave a purer fraction (Q 3).

Q 1 was free from maltase, amyloses and fatty acid. With amylose as substrate, Q 1 differs from the amylases in that, whilst causing a 90 *per cent.* diminution of the absorption value (A.V.) of amylose, only 2 *per cent.* (as maltose) of reducing end-groups were released. The enzyme had an optimum p_H of 7.0 and the unusually low optimum temperature of $21 \pm 1^\circ C$. The criterion of activity was taken as the diminution of A.V. (6800 Å.) of amylose (blue value 1.04) produced in unit time by the enzyme acting under optimal conditions of temperature and p_H . At room temperature Q-enzyme solutions rapidly lost their activity. The enzyme was also inactivated by alcohol precipitation. The best storage results were attained by freeze-dried preparations of Q 1, which could be kept for long periods at $0^\circ C$. with less than 30 *per cent.* loss of activity. Q 3, however, lost 90 *per cent.* of activity on freeze-drying, a result tentatively ascribed to inactivation by acidity developed during drying and which is counteracted in the more stable Q 1 fraction by buffering substances present as impurities. C. R.

Action of Q-Enzymes on Starch and its Components. S. A. BARKER, E. J. BOURNE and S. PEAT (*J. chem. Soc.*, 1949, 1712-1717). A dual function has been ascribed to Q-enzyme (this *Journ.*, 1946, 152): (1) the catalysis, in conjunction with phosphorylase, of amylopectin synthesis, from glucose-1-phosphate; (2) the conversion of amylose into amylopectin, without liberation of reducing sugar. With the isolation of Q-enzyme (see previous abstract), confirmation of the second function was obtained. Studies were made of the action of p_H 7.04 and $20.5^\circ C$. of Q-enzyme on potato starch and the

amylose and amylopectin prepared from it. The action was followed by determinations of reducing power and absorption value (A.V., 6800 Å.) of the iodine complex on aliquots withdrawn from the reaction mixtures. Acting on amylose of blue value (B.V.) 1.04, Q-enzyme effected a total decrease of 87 per cent. in A.V., the wavelength of peak absorption shifting from 6400 to 5200 Å., the latter value being close to that of waxy maize starch. At the same time, the reducing power developed only corresponded to 2 per cent. maltose conversion. The results indicated the formation of a branched from a linear chain. This was further demonstrated by the simultaneous action of Q-enzyme and β -amylase (soy bean) on amylose. β -amylase alone effected a 90.2 per cent. conversion to maltose in 210 min., but only a 74.2 per cent. conversion in the same time when Q-enzyme was also present. Prior incubation of the amylose with Q-enzyme before the action of β -amylase gave still lower maltose conversion figures.

The question whether amylopectin was built by Q-enzyme from intact amylose chains, or from chains which had first undergone cleavage by the enzyme, was decided by studying the initial rate of maltose production from amylose by weak β -amylase solutions in the presence of Q-enzyme. The speed of β -amylolysis depends upon the number of chain-ends available, so that chain cleavage would be reflected by a greater rate of amylolysis as a result of the combined action of β -amylase and Q-enzyme than when β -amylase acted alone. An increase in the initial rate of maltose production was, in fact, found. Pretreatment of the amylose with Q-enzyme before the action of β -amylase proved even more effective. Despite this initial increase in rate of β -amylolysis due to the presence of Q-enzyme, a decreased limiting conversion to maltose was recorded. Thus Q-enzyme effected both a cleavage of amylose chains and a cross-linking of the fragments.

Red-staining polysaccharides were isolated in yields of over 90 per cent. by the action of Q-enzyme on starch, amylose and amylopectin, from potato and on waxy maize starch. The products were protein-free and readily soluble in hot water. Under acid hydrolysis, each gave more than 93 per cent. conversion to glucose, while with β -amylase, each gave a limit dextrin and 52-57 per cent. maltose. The B.V. of the product was always less than that of the parent starch and light absorption curves of the iodine complexes closely followed the curve for natural amylopectin. Thus, the four products, although not identical, possessed the branched amylopectin structure. A practical result of the work is that there is now available, in powder form, an enzyme preparation which will convert

amylose or natural starch wholly into non-retrograding, water-soluble amylopectin. C. R.

Enzymic Hydrolysis of Amylopectin. Isolation of a Crystalline Trisaccharide Hendeca-acetate. M. L. WOLFROM, L. W. GEORGES, A. THOMPSON and I. L. MILLER (*J. Amer. chem. Soc.*, 1949, 71, 2873-2875). Past work on oligosaccharides found in starch hydrolysates has depended on characterization of syrupy products (this *Journ.*, 1942, 149; 1944, 250). A crystalline trisaccharide derivative has now been obtained from enzymic hydrolysates of waxy maize starch. Hydrolyses of the starch (almost 100 per cent. amylopectin) were carried out at 55° C. for 5 days with a commercial barley malt diastase. After boiling to inactivate the enzyme, the digest was freed from inorganic material by passage through Amberlite resins, and the filtrate concentrated *in vacuo* to a syrup, which was finally dehydrated by removal of the water *in vacuo* as the water-ethanol azeotrope. 25 gm. of the resultant syrup was acetylated at 120-130° C. with 15 gm. anhydrous sodium acetate and 150-200 ml. acetic anhydride, then cooled and poured into 2,500 gm. of ice and water. The crude acetates separating were washed with water on a filter and air-dried. Chromatographic separation of the acetates was carried out by placing a solution of 5 gm. of the crude acetates in 75 ml. benzene on a Magnesol-Celite column and developing with benzene-ethanol (100 : 1 by volume). The major zone near the bottom of the column consisted of β -maltose octa-acetate. A second zone, near the middle, was sectioned and eluted with acetone. Removal of the solvent left a syrup which crystallised from ethanol and was recrystallised from the same solvent in yields of 0.03-0.4 gm. This material had m.p. of 134-136° C. and $[\alpha]_D^{25} + 86^\circ$ (chloroform) whilst its elemental analysis and molecular weight agreed closely with that for a trisaccharide hendeca-acetate. No β -isomaltose octa-acetate (this *Journ.*, 1949, 254) was found but two other zones above the trisaccharide zone, on elution with acetone, yielded syrups which failed to crystallize on long standing.

C. R.

Hydrolysis of α -1,6-Glucosidic Linkage in isoMaltose by Culture Filtrate of *Aspergillus niger* NRRL 330. H. M. TSUCHIYA, E. M. MONTGOMERY and J. CORMAN (*J. Amer. chem. Soc.*, 1949, 71, 3265). Hydrolysis of the linkage between the two glucose residues in isoMaltose is of interest because the same linkage in the amylopectin fraction of starch resists hydrolysis by the more common amyolytic enzymes. By submitting 0.06 M solutions of isoMaltose monohydrate to the action of

different concentrations of a culture filtrate of *Aspergillus niger* NRRL 330 at p_H 4.4 and temperature 50° C. (122° F.), the authors have achieved as much as 90 per cent. hydrolysis of the isomaltose. The glucose produced was estimated by fermentation with yeast, and identified after isolation as crystalline α -D-glucose monohydrate; $[\alpha]_D^{20} + 47.8$ (4 per cent., in water), m.pt. 82.8° C. (177° F.). A. A. D. C.

Pectic Enzymes of *Byssoschlamys fulva*.

G. H. BEAVEN and F. BROWN (*Biochem. J.*, 1949, **45**, 221-224). The identity of the pectic enzymes involved in the disintegration of fruit tissue by the mould *B. fulva* was investigated. Three enzyme sources were used: (1) infected bottled fruit; (2) Czapek-Dox pure culture filtrates; (3) extracts of the mycelium of the mould grown on various media. Protopectinase activity was detected by its macerating action at 37° C. on potato discs cut to standard size (12 $\times$ 0.5 mm.) by a microtome. Pectin methoxylase activity was measured by titration with alkali of the carboxylic groups set free from commercial citrus pectins and from polygalacturonide methyl ester. The two latter substrates, and an enzymically prepared pectic acid, were used for studies of pectinase, activity being determined viscometrically in terms of unchanged substrate or by reducing power in terms of D-galacturonic acid.

Protopectinase activity was demonstrated in five or six specimens of infected bottled fruits and in four of seven Czapek-Dox cultures. Two of the inactive cultures were anaerobic with submerged mycelia. There was some indication that asparagine in the medium enhanced production of the enzyme. Maximum activity was observed at p_H 4-5, complete inactivation at 65° C. taking place at p_H 4 in 5-10 min. *B. fulva* appeared unique in that pectin methoxylase was absent from all sources examined, although a pectinase complex was present. Activity of the complex showed no optimum over the range p_H 3-5. It caused an initial rapid fall in viscosity of pectin solutions, not accompanied by a significant increase in reducing power, indicating the presence of a disaggregating enzyme, but no glycosidase. This work supports the view that the large pectin molecule consists of relatively small polyuronic acid units joined by non-glycosidic linkages. C. R.

Proteins. G. MASTENBROEK (*Bull. Assoc. anc. Etud., Louvain*, 1949, **45**, 1-15). This is a survey of the techniques used in modern protein research. After a brief discussion of the importance of proteins in nature, the author describes the means by which they may be isolated. Chemical methods consist of submitting a solution of the protein to either change of temperature, change

of p_H , the ionic forces set up by certain multivalent ions, the "salting-out" effect of salts such as ammonium sulphate, or the effect of different concentrations of methyl or ethyl alcohol. Salting-out tends to cause denaturation, but this can be prevented by working near the freezing point of the solution. Physical methods are proving more successful, and the techniques of electrophoresis and the ultra-centrifuge are described in more detail. The former is based on the fact that every colloidal particle has, under given conditions of p_H , temperature and ionic force, a specific mobility when its solution is placed in an electric field of unit strength. This mobility is determined by measuring the speed of movement of the particle towards its oppositely-charged electrode, and dividing the speed by the field strength (which is the electrode potential divided by the distances between the electrodes). With few exceptions, each protein has a different specific mobility, and in the course of electrophoresis of a mixture individual proteins separate into a number of homogeneous layers. The layers have different refractive indices, and their optical analysis enables a curve to be drawn in which each maximum point denotes a protein and the height of each maximum denotes its respective concentration. The components of a mixture of two proteins of different specific mobility can be isolated by means of electrophoresis. Where mixtures of proteins of the same electrical charge (*i.e.*, the same specific mobility) are encountered, the ultra-centrifuge provides a means of separation by making use of the different sedimentation rates of colloidal particles of different size and shape. From a measurement of its sedimentation rate under known conditions of concentration, temperature and centrifugal force, the molecular weight of a protein can be calculated. This technique has already been applied to studies of the proteins in barley and yeast. A. A. D. C.

Protein Foams Obtained by Bubbling.

W. C. THUMAN, A. G. BROWN and J. W. MCBAIN (*J. Amer. chem. Soc.*, 1949, **71**, 3129-3135). Egg albumin and salmine (protamine) sulphate were used in this study, and curves have been constructed between foaming capacity and p_H , under various conditions of dispersion, and in absence and presence of certain salts. Foaming capacity was determined by placing 1 c.c. of the liquid on the porous plate of a modified Stiepel-type foam meter, passing air through the plate at the rate of 16 c.c. per min., and measuring the maximum volume of the foam produced. Determinations were made in replicate. The results show that the proteins produce maximum foam slightly on the acid side of the isoelectric point (p_H 4 for egg albumin, p_H 10 for protamine). The albumin also shows

high foaming capacity at very high and very low p_H values. The maximum foaming capacity is the same whether the albumin was originally dispersed at p_H 5 or dissolved in potassium or barium hydroxide at p_H 10, but its position is slightly shifted. The maximum is enhanced by dissolving the egg albumin in 0.001 M acetic acid. Albumin merely dispersed in water at p_H 5 does not foam between p_H 7 and 11, but if it is dissolved in potassium or barium hydroxide it foams freely within this range. Alkaline earth ion or salt greatly promotes foaming in the alkaline range, but may minimize it on the acid side. There appears to be a significant correlation between denaturation and maximum foaming, and only under these conditions is the protein adsorbed at the air-solution interface as a fully extended coherent film, probably

of the "gel" type. At maximum foaming the air-solution surface area is estimated to be of the order of 1 sq. metre for each 5 mgrm. of albumin in the original solution. It would appear that a condition of maximum foaming is that there should be a minimum net charge on the protein complex, either by suppression of ionization of the protein at very high or very low p_H values, or at the isoelectric point due to "self-neutralization," or at intermediate p_H values by neutralization of free carboxy- or amino-groups by kations or anions respectively. The results of the study, seen in the light of previous knowledge, indicate that different proteins follow a general pattern in their foaming behaviour, and that this pattern is similar to that shown by other protein properties.
A. A. D. C.

PATENTS

Method for Racking and Drawing-off Beer under Sterile Conditions and Protecting the Liquid from Air. J. E. L. LEPPERRE (Brit. Pat. No. 615,258, 17th May, 1946). A cask is provided with a collapsible lining of rubber or similar material and also a side valve by means of which compressed air may be introduced between the cask wall and the lining. This lining is collapsed by passing a current of compressed air through the valve and a bung hole with a valve at its lower end fitted over the neck of the lining is connected with a feed pipe conveying the beer. After filling, the feed pipe is removed and replaced by a closing plug. The cask is emptied by removing the plug, inserting a draw-off cock and passing compressed air through the side valve, when the pressure exerted on the lining forces the beer out of the cask. T. J. W.

Recovery of Valuable Constituents from Yeast. Schwarz Labs., Inc. (Brit. Pat. No. 615,891, 20th Nov., 1944). Pressed yeast is mixed with about 5 *per cent.* of its weight of 50 *per cent.* sodium hydroxide solution and heated for 1 hr. at 60–65° C. After dilution with water, concentrated sulphuric acid is added to yield a p_H value of 4.5–4.7, the mixture is boiled for 20 min., cooled and filtered to remove protein and insoluble cell walls. Sodium hydroxide in an amount equal to 0.2–0.5 *per cent.* of the weight of yeast is added and the protein is removed by filtration. The reaction of the filtrate is adjusted to p_H 5.5, boiled and copper sulphate is added, followed by sodium bisulphite solution until the p_H value is 3.0. The precipitate formed containing the nucleotides is separated, washed and treated as in Brit. Pat. No. 590,995.

T. J. W.

REVIEWS

Chemistry and Technology of Wines and Liquors.

By KARL M. HERSTEIN and MORRIS B. JACOBS. Pp. xii + 436. New York: Van Nostrand Co., Inc. (London: Macmillan & Co., Ltd.) 1948. Price 36s.

A dearth of technical books on the fermented beverages written in English is being slowly made good, although so far as wines and spirits are concerned, it has been left to American authors to make good the deficiency. Since Nettleton's famous treatise little has appeared on potable spirits save the long unobtainable book by Simmonds. This new book is a useful contribution to our needs. The authors are a consulting and an official chemist who are well qualified to discuss the scientific and, in particular, analytical aspects of the subject. Yet it is just here that such shortcomings as there are make their appearance as will be referred to later. A previous edition, by Herstein and Gregory (1935) has been largely rewritten, the revision being mainly concerned with the early chapters on theoretical aspects of fermentation, newer work on the ageing of whiskey and recent advances in production and analytical methods.

After four chapters on scientific and theoretical matters, sugars and starch, enzymes, raw materials, and fermentation organisms, the authors pass on to the various technical processes. The first of these is the production of yeast for use in the distillery, it does not refer to baker's yeast. The use of molasses in this connection is not discussed, but the grain mash process is adequately described. Wine starters, including pure yeast culture for this purpose, receive brief treatment. Malting is too briefly treated to be of use to the British worker and is concerned with American barleys, malt and methods.

A chapter on distillation is of general interest the principles being clearly described and many designs of still furnished with illustrations. An interesting chapter on the history of whiskey in Great Britain with its record of restrictive legislation and its evasion through recent centuries is followed by much information about the legal aspects—which so far as U.S.A. is concerned is adequate. Indeed, there is throughout the book an inevitable predominance given to American methods but so far as whiskey is concerned, there is, except for legal matters, some attention paid to Scotch and Irish production. Here both pot and patent still spirits are produced and blended, and art still plays a big part. In U.S.A. only the patent or continuous still is used.

Chapters on ageing and blending are full of information and modern mechanical, physical and other aids to ageing such as the use of ultra-violet and other rays, activated carbon, oxidation, etc., designed to save time, are briefly described.

The book is a regular compendium of the various spirits, liqueurs, cordials, etc., and this part would be of considerable interest to the layman, especially the chapters on wines and liqueurs. They deal with the classification, production and uses of the beverages—there is little or no chemistry here, but such matters as formulae for a number of well-known liqueurs are to be found.

There are comprehensive tables of analyses of wines and spirits, including Scotch and Irish whiskeys, but they are almost all old results dating back to early in the century. There is discussion of the interpretation of analytical results but little about other, non-analytical, methods of judging spirits, although they are most important. The methods of analysis given are the Official and Tentative Methods of the A.O.A.C., accompanied by the necessary analytical tables of reference.

Some mistakes occur in purely scientific matters. The type adopted for structural formulae of sugars is ambiguous and some formulae are actually incorrect. Invertase is referred to as diastase and maltase described as β -glucosidase. The organism *Saccharomyces mycoderma* is not known to us, at least not by that name. The chapters on the chemistry of alcoholic fermentation and on the yeasts are, however, very good simple accounts of their subjects.

There are some 50 odd tables, 60 illustrations, a general index, but no bibliography.

R. H. HOPKINS.

Die Rohstoffe der Gärungsindustrie (Raw Materials of the Fermentation Industries).

By H. VOGEL, Dr. phil., Dipl. Ing. Pp. vii + 167. Basel: Wepf & Co. 1949. Price 18.00 Swiss francs.

If the test of a book is that it shall fulfil literally the promise implicate in its title, then this volume succeeds admirably as it discusses in reasonable detail a surprisingly wide range of materials which are, or could conceivably be used as, raw materials in fermentation processes. A section on sugary

products deals with sugar beet (chiefly from the Continental point of view) and cane, and the manufacture therefrom of sucrose with production of molasses; sources of whey and lactose production are also considered. Starchy materials allow a discussion of potatoes and a wide variety of cereals. The compositions of these materials are given usually in the form of average data—a not very satisfactory proceeding, perhaps, though it is difficult to suggest what more could be done in a volume of this size; nevertheless, it may be pointed out that much information is available concerning the nitrogenous composition of barley at least, and a discussion of nitrogen distribution seems worthy of inclusion. Sweet potatoes, yams, manihot, Jerusalem artichokes, chicory, dahlias, Kog-Sagys—all these are treated in some detail. A further section of interest brings together in a useful way a collection of data relating to the use of wood and other cellulosic materials as sources of fermentable sugars; saccharification methods and the use of sulphite liquor and other waste products are all included. Many liquors for fermentation require the addition of inorganic salts as sources of essential elements, and this aspect is covered by the inclusion of a final section devoted to the sources, and methods of manufacture, of suitable salts, this giving a somewhat unexpected measure of completeness of treatment. Wherever technical processes are described in this book, only outline descriptions are given, but sufficient is usually provided to allow the principles of operation to be readily understood.

Thus, so far as it goes, the book is very good. It might be suggested that it should go rather further. In particular, more than a mere list of enzymes in a particular raw material, *e.g.*, potatoes or barley, would seem to be desirable. The importance of these enzymes might be indicated—the relation of oxidizing enzymes to the colour of potato products, the function of cereal enzymes in making possible the use of cereals as sources of fermentable sugars; a case could be made out for including the preparation of malt, especially as space is found for including the preparation of sucrose and starch. Again, 268 references to the literature are given. It is a pity that original sources are not quoted in every case; instead, in a number of instances, reference appears to be made (without explanation) to an Abstract system.

These suggestions are intended to be constructive. There is much that is admirable in this book, which already provides an extremely valuable compilation of data; it is felt that its utility would be even greater if attention could be given to the points mentioned

I. A. PREECE.

The Food Manufacturing Industry in Germany during the Period 1939-1945. British Intelligence Objectives Sub-Committee Report No. 14. Ed. H. G. HARVEY. Pp. 134. London: His Majesty's Stationery Office. 1949. Price 2s. 6d.

At the end of World War II, a survey was made of most branches of the German food manufacturing industry by various intelligence committees and agencies. This Report is a critical summary of the information contained in some 200 reports drawn up by these bodies. To the busy food technologist, who can ill-afford the time necessary to sift the mass of detail spread over 200 documents, this Report presents, in a readily available form, a great deal of interesting material. The chief aim of the Report is "to give special mention to novel features and to suggest . . . applications and improvements to their British counterparts."

The Report consists of 19 sections, the last of which is devoted to matters of interest to the Australian food industry, together with an index and bibliography. From the point of view of most readers of this *Journal*, perhaps the most interesting sections are those on the production of wood sugar and fodder yeast, the baking industry and the soft drinks industry. Contrary to a common impression, the production of alcohol and fodder yeast in Germany from cellulosic material, whilst of significant proportions, was small, although plans had been laid for increased production from 1944 onwards. Again, there was no large-scale production of fats by micro-organisms in Germany during the war. In the field of baking, the production of baker's yeast followed conventional practice, although some development took place of the use of X-ray mutants of a strain of yeast used for the production of sour rye bread. The brewing of beer is not dealt with in the Report.

One general conclusion seems to be that, rather than lagging behind in the technique of food production, Britain was, in fact, more up-to-date and more operationally efficient than Germany, exceptions to this conclusion occurring notably in the soft drinks industry and in the production of wood sugar and fodder yeast. However, the Report brings out many new features which may prove useful in application in this country, particularly in the field of plant equipment and in that of the production of egg albumen substitutes.

In a Germany in which, for many years, guns had taken priority over butter, it is not surprising to read that, in the German food industry, relatively little use was made of scientific staff, either in the factory or in research institutes, and that liaison between research and industry was poor.

C. RAINBOW.

